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Early Markers of Dysfunction in Frontotemporal Dementia

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Abstract

Frontotemporal Dementia (FTD) is a neurodegenerative disorder that results in atrophy within the frontal and/or temporal lobes. Clinically, patients with FTD present with progressive deterioration in behaviour and/or language abilities. FTD has a strong genetic component with approximately 40% of patients reporting a family history. Specifically, mutations in microtubule-associated protein tau (*MAPT*), progranulin (*GRN*) and expanded repeats in the chromosome 9 open reading frame 72 (*C9orf72)* are the main genetic causes of FTD. Currently, no disease-modifying treatments exist, and off-label medications have been used for symptomatic management of behaviours. Substantial progress has been made to understand the underlying pathology of the disease and clinical trials targeting FTD are in progress. As clinical trials begin, the identification of disease markers will be critical to measure treatment effects, indicate when treatments should be initiated and serve as potential targets for treatments. Therefore, there is a critical need to identify disease markers in FTD.

The present work aimed to elucidate behavioural, structural, and functional changes in FTD from the preclinical to the symptomatic disease stage. Study I delineated the initial symptoms in patients with genetic FTD and in at-risk gene mutation carriers (preclinical mutation carriers and non-mutation carriers). This study revealed gene-specific patterns of initial symptoms during the preclinical and symptomatic disease period. Study II examined the brain's ventricular volumes in genetically at-risk mutation carriers. Preclinical mutation carriers exhibited larger ventricular volume (i.e. greater neuronal atrophy), relative to biologically related mutation non-carriers. Study III delineated the functional neural correlates underlying disinhibition and behavioural flexibility in patients with FTD. Relative to healthy controls, patents with FTD exhibited decreased activity within the ventral and dorsal lateral regions of the

prefrontal cortex. This study reveals that patients with FTD exhibit aberrant neural functioning relative to healthy controls in a task indexing behavioural flexibility.

Overall, this work suggests that behavioural and neuroanatomical disease-alteration occur during the preclinical disease stage and functional neural deficits underlying behavioural difficulties can be detected in symptomatic patients. These results may be applied to future clinical trial designs to assess the efficacy of treatments and determine potential treatment targets.

Keywords

Frontotemporal Dementia, biomarkers, clinical trials, design-modifying treatments, symptomatic treatments, chromosome 9 open reading frame 72, *C9orf72*, progranulin, *PGRN*, Microtubule-associated protein tau, *MAPT*, preclinical, disease onset, ventricular volume, reversal learning, symptoms, functional magnetic resonance imaging

Summary for Lay Audience

Frontotemporal Dementia (FTD) is a specific type of dementia that leads to brain tissue loss. Patients with FTD demonstrate behaviour and/or language problems including disinhibition, loss of empathy, difficulty attributing the correct meaning to words and word production. Although no disease-modifying treatments exist, substantial progress has been made to understand the pathology of the disease. As clinical trials begin, the identification of biomarkers will be essential to: (1) indicate disease presence and progression, (2) measure treatment effectiveness, (3) indicate when treatments should be administered, and (4) lead to the selection of treatment targets. Thus, there is a critical need to identify disease markers of FTD.

The present thesis examined the behavioural, structural, and functional changes in FTD from the preclinical (prior to disease occurrence) to the disease stage. Study I examined the initial symptoms in patients with FTD and in preclinical mutation carriers (individuals with the disease-causing mutation but who do not yet meet the criteria for the disease), and non-mutation carriers (individuals who are not carrying the disease-causing mutation). This study found that patients' initial symptoms differ based on the underlying genetic mutation, and preclinical mutation carriers show unique symptoms relative to mutation non-carriers. Study II examined the volumes of the brain's ventricles (cavities filled with cerebrospinal fluid) in genetically at-risk mutation carriers. Preclinical mutation carriers exhibited greater brain tissue loss relative to biologically related mutation non-carriers. Study III assessed the functional neural correlates underlying poor behavioural flexibility in patients with FTD. Relative to healthy controls, patients with FTD showed decreased activity within the regions of the frontal lobe known to be important for appropriate decision-making.

Overall, this work suggests that disease-alterations in behaviour and brain structure occur and can be detected during the preclinical disease stage prior to the onset of the disease. Furthermore, this work also demonstrates that problems in brain function related to behaviour flexibility can be detected in patients with FTD. These results may inform the selection of disease markers for clinical trial designs.

Co-Authorship Statement

Chapter 1, the introduction, and Chapter 5, the general discussion were written by Tamara Tavares with input from Dr. Derek Mitchell and Dr. Elizabeth Finger. Chapter 2 was written by Tamara Tavares with input from the co-authors. GENFI consortium members were involved in experimental design and data collection. Statistical analysis was conducted by Tamara Tavares and supervised by Dr. Brenda Coleman and Dr. Elizabeth Finger. Chapter 3 was written by Tamara Tavares with input from the co-authors. GENFI consortium members were involved in experimental design and data collection. Statistical analysis was conducted by Tamara Tavares and supervised by Dr. Garrick Wallstrom, and Dr. Elizabeth Finger. Chapter 4 was written by Tamara Tavares with input from Elizabeth Finger and Derek Mitchell. Elizabeth Finger and Derek Mitchell were involved in the experimental design. Tamara Tavares completed the statistical analysis. Kristy Coleman, Julia MacKinley Sarah Jesso and Mika Ohtsuka were involved in the data collection.

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Chapter 1: Introduction

1.1 Frontotemporal Dementia

Frontotemporal Dementia (FTD) is a heterogenous neurodegenerative disorder resulting in progressive deterioration in behaviour and/or language abilities. FTD includes three core subtypes: behavioural variant (bvFTD) and two primary progressive aphasias (PPA), semantic variant PPA (sv-PPA) and nonfluent/agrammatic variant PPA (nfvPPA). Related disorders include FTD with motor neuron disease (FTD-MND), progressive supranuclear palsy syndrome (PSP-S), and corticobasal syndrome (CBS). Although distinct clinical subtypes exist, the presenting phenotype often converges with other sub-types as the disease progresses [1,2], which may complicate diagnosis if not recognized early.

1.2 Epidemiology

FTD is the second most prevalent early onset dementia occurring before the age 65 [3], with a prevalence of 18 to 36 per 100,000 in individuals 45-64 years of age [4]. FTD is primarily diagnosed between the ages of 45-65, with approximately 10% of cases occurring in individuals younger than 45 years of age [4], and the prevalence more than doubling amongst those older than 65 years of age [4,5]. Importantly, though the prevalence is likely underestimated due to lack of expertise by primary care physicians with limited expertise in behavioural neurology [4], and the absence of validated biomarkers to distinguish FTD from other neurologic and psychiatric disorders [6].

1.3 Burden

FTD causes a substantial economic and social burden to the caregiver, patient and society. The mean age of disease onset occurs during a time when an individual's contribution to

society is at its greatest, as many patients are in their prime earning years and have dependent children [7]. Overall household income decreases significantly after diagnosis, and the total annual per-patient cost is nearly two times higher than the reported cost for Alzheimer's Disease (AD) [7]. In addition to the contribution to society, the burden to the caregiver is substantial: 67% of caregivers of patients with FTD reported a notable decline in their own health and 53% reported increased personal health care costs [7]. Relative to carers of patients with AD, caregivers of people with FTD report greater burden [8,9], and experience twice the levels of depression, even after controlling for age and duration of symptoms [10].

1.4 Prognosis

Across the syndromes, the mean survival time of patients with FTD is approximately 7-8 years from diagnosis [11]. In a sample of 124 patients, the most common cause of death was respiratory system disorder (27%), circulatory system disorder (19%) and cachexia (14%). Additionally, 11.5% of patients died from cancer, and in 11.5% of patients, the cause of death was unknown [12]. Having genetic FTD has been found to be associated with a shorter survival time [13]; other demographic characteristics including age at illness onset or severity of dementia at the time of diagnosis have not been found to be associated with survival [12,14].

1.5 What causes FTD?

1.5.1 Genetic FTD

FTD has a strong genetic component with approximately 40% of patients reporting a family history and approximately 10% demonstrate an autosomal dominant pattern of inheritance [15]. Importantly, the exact percentage of cases with a family history may vary as inaccurate reporting

of family history of FTD may be attributed to psychiatric disease, another dementia or other diagnoses. Heritability varies across the clinical subtypes with the bvFTD phenotype showing the strongest heritability and the language variants showing the least [15-17]. Mutations in three genes have been shown to be the most frequent genetic causes of FTD including microtubule-associated protein tau (*MAPT*) [18,19], progranulin (*GRN*) [20,21], and expanded repeats in the chromosome 9 open reading frame 72 (*C9orf72)* [22,23] (Table 1.1). Together, these genes account for 5-10% of all FTD with some variability depending on the case series [24].

Table 1.1: Summary of FTD-related genetic mutations

FTD gene	Frequency[25,26]	Age at symptom onset (years)[27]	Disease duration (years)[27]	Pathology[26]
MAPT	Familial: 5-20% Sporadic: 0-3%	49.5 Range: 17-82	9.3 Range: 0-45	Tau protein deposits
GRN	Familial: 5-10% Sporadic: 1-5%	61.3 Range: 25-90	7.1 Range: 0-27	Accumulation of TAR DNA-binding protein 43 (TDP-43)
C9orf72	Familial: 21% Sporadic: 6%	58.2 Range: 20-91	6.4 Range: 0-36	Accumulation of TAR DNA-binding protein 43 (TDP-43)

1.5.1.1 Microtubule-associated Protein Tau (MAPT)

The *MAPT* gene located on chromosome 17 was the first pathological mutation found to cause FTD [18,19]. To date, 63 different *MAPT* mutations have been identified in patients with FTLD [24]. Patients often present with bvFTD [28], though some may develop semantic impairments and parkinsonism as the disease progresses [29]. Importantly, significant variability remains in the presenting phenotype, even between families carrying the same mutation [30].

1.5.1.2 Progranulin (GRN)

Mutations in the *GRN* gene lead to haploinsufficiency, resulting in reduced levels of progranulin protein, a growth factor involved in regulating developmental events, inflammation and wound repair [31]. To date, 114 different *GRN* mutations causing FTD have been identified [24]. There is considerable variability in the age of disease onset, even for patients carrying identical *GRN* mutations [32]. As well, the presenting phenotype in the setting of a *GRN* mutation is quite heterogeneous with patients exhibiting Alzheimer's Disease, Corticobasal Syndrome, or nfvPPA, with the majority of patients exhibiting bvFTD phenotype [27,33].

1.5.1.3 Chromosome 9 open reading frame 72 (C9orf72)

Pathogenic hexanucleotide (GGGGCC) repeat expansion of the *C9orf72* gene is a major genetic cause of FTD [22]. The normal hexanucleotide repeat size is quite variable across individuals and the smallest repeat size to confer risk is currently unknown [34]; however, most consider > 30 repeats as pathogenic [33]. The most common phenotype is bvFTD, followed by mild cognitive impairment (MCI), then FTD with ALS [28].

1.5.2 Sporadic FTD

Approximately, 60% of patients report no family history of FTD and are considered "sporadic" [15]. A pathogenic variant is found in 5% of sporadic cases with mutations in the *C9orf72* being the most frequent [26,28]. Furthermore, previous head trauma has been found to be a significant risk factor for the development of sporadic FTD when compared with age and gender-matched controls [35]. Although the majority of familial and sporadic cases arise from

different underlying pathologies and genetic factors, both forms show similar behavioural, cognitive and motor measures [36].

1.6 FTD Syndromes

Although there are three distinct clinical variants of FTD, each presenting with a unique phenotype, the clinical profile within each variant is quite heterogenous. Nevertheless, the subtypes are categorized under the broader name of FTD due to the shared clinical features, high degree of anatomical overlap in structural and functional brain imaging and overlap in pathologies [37]. Furthermore, as FTD progresses, the symptoms of the three variants converge as the focal neural degeneration progresses through the networks of the frontal and temporal lobes [38].

1.6.1 Behavioural Variant FTD (bvFTD)

bvFTD is the most common variant and accounts for approximately 60% of cases [39]. The clinical symptomatology of bvFTD is quite heterogeneous and is characterized by a deterioration of behaviour and/or cognition that begins insidiously and progresses gradually over time. The core diagnostic features include behavioural disinhibition, apathy, loss of sympathy, hyperorality, and/or perseverative or compulsive behaviours [40]. Additionally, patients may also demonstrate frontal and/or anterior temporal atrophy, hypoperfusion or hypometabolism [40].

1.6.2 Semantic-variant Primary Progressive Aphasia (sv-PPA)

Sv-PPA accounts for 20% of FTD cases [39] and is clinically characterized by a loss of semantic knowledge resulting in word comprehension difficulties and anomia [41]. Early during the disease, comprehension for high frequency words is intact but patients may experience difficulty understanding low frequency words. Patients may also present with surface dyslexia and dysgraphia where atypical spelling or pronunciation of words are regularized, for example island is pronounced "is" – "land" [41]. Imaging features include hypoperfusion, hypometabolism, and/or atrophy of the dominant anterior temporal lobe, with common involvement of the non-dominant side as well [41]. Adding to the complexity, as the atrophy progresses, behavioural symptoms emerge including emotional withdrawal, disinhibition, apathy [42].

1.6.3 Nonfluent/agrammatic-variant Primary Progressive Aphasia (nfvPPA)

nfvPPA accounts for 25% of all FTD cases [39]. The hallmark features of nfvPPA are the presence of agrammatic and effortful speech, and apraxia of speech [41]. Patients may make inconsistent phonemic errors including deletions, distortions, insertions, substitutions or transpositions of speech sounds [41]. As the disease progresses, patients will make grammatical and spelling errors and comprehension of complex sentences is reduced [43]. Imaging-supported diagnosis involves predominant left posterior fronto-insular atrophy or hypoperfusion or hypometabolism [41].

1.7 Current Challenges

1.7.1 Diagnostic Delay and Misdiagnosis

Although the current diagnostic criteria demonstrates good sensitivity to detect FTD [40], the vast heterogeneity in clinical presentation across and within syndromes, and the significant overlap in symptom dimensions with other diseases, poses a challenge in accurately detecting patients early. There is a delay of nearly 6-7 years before patients receive an accurate diagnosis [42,44]. As well, patients with FTD, especially those with bvFTD subtype, are commonly misdiagnosed with a psychiatric illness [45,46], leading to incorrect disease prognosis and unnecessary treatments. Ultimately, the identification of FTD-related biomarkers sensitive to early disease onset would help mitigate this diagnostic challenge.

1.7.2 Current Treatment Interventions

At present, there are no U.S. Food and Drug Administration (FDA) or Health Canada approved therapies for FTD, and no treatments exist that can stop or slow the progression of this disease. Off-label medications have been used for symptomatic management of behaviours; however, there is little evidence from randomized, placebo-controlled trials supporting their use [47]. Nonpharmacological interventions targeting environment adaptation, behaviour strategies and caregiver training and education have also been employed [48]. Although these interventions alleviate some of the caregiver burden, they do not modify the course of the disease as they do not target the underlying pathology of FTD [6]. Currently, some of the first clinical trials targeting FTD are in progress or anticipated (Table 1.2). Importantly though, one key remaining challenge in clinical trial design for FTD is the vast heterogeneity of clinical symptoms, even in

individuals with the same mutation or underlying pathology, making it difficult to measure treatment effects and identify appropriate outcome measures [6].

Table 1.2: Current drugs in clinical trials for FTD

Drug	Population	Status	Phase	Identifier
AL001	Healthy participants and carriers of GRN	Recruiting	Phase 1	NCT03636204
AL001	Carriers of GRN or pathogenic forms of C9orf72	Recruiting	Phase 2	NCT03987295
AL001	Carriers or at-risk carriers of GRN	Not yet recruiting	Phase 3	NCT04374136
Metformin	ALS/FTD with C9orf72	Recruiting	Phase 2	NCT04220021
Novolin-R insulin	FTD	Recruiting	Phase 2	NCT04115384
Syntocinon	Probable FTD	Recruiting	Phase 2	NCT03260920
AADvac1	Non-fluent PPA	Active, not recruiting	Phase 1	NCT03174886
Lithium carbonate	Participants with a diagnosis of bvFTD, sv-PPA or nfv-PPA	Recruiting	Phase 2	NCT02862210

Based on clinicalTrials.gov, accessed May 25 2020

1.8 Knowledge Gaps for Optimization of Clinical Trials in FTD

1.8.1 The Need for Biomarkers

With the development of symptomatic and disease-modifying treatments, the identification of biomarkers will be critical for clinical trial efficiency. Biomarkers may facilitate earlier detection of symptoms and diagnosis prior to irreversible neuronal loss. Furthermore, by improving diagnostic accuracy, recruited study populations will be more homogenous and thus, the necessary sample size needed to detect treatment effects will be reduced [49]. As well, the identification of biomarkers that can predict disease progression and symptom severity may act as outcome measures to indicate when treatments should be initiated and to assess treatment efficacy. To date, promising fluid biomarkers including cerebrospinal fluid and serum/plasma levels of neurofilament chains and progranulin are being explored [6,50]. As well, the

quantification of individualized patterns of atrophy has been shown to be another potential candidate [6,51]. Importantly, though, no validated markers for FTD have been established for clinical trials [52,53]. Consequently, it is critical to identify potential biomarkers that can be applied to develop efficient clinical trials, monitor disease progression, and evaluate treatment effects.

1.9 Early Clinical Features in FTD

Prediction of the initial symptoms of FTD in an individual patient remains a critical challenge both for clinical trial design and diagnosis of FTD. The initial symptoms are a critical milestone that will require correlation with other potential biomarkers. However, in line with the differing diagnostic criteria for the sub-types, the predominant initial symptoms in FTD differ according to the clinical syndrome [54,55]. Importantly though, there remains substantial heterogeneity in the initial symptom endorsement even within an FTD subtype. For example, patients with bvFTD may also endorse memory, language, or other cognitive symptoms as the initial symptom(s). Likewise, patients with SD may also report behavioural, memory and other cognitive problems as the initial symptom(s) [54,55]. Furthermore, the initial symptoms of FTD reported by caregivers are not always congruent with the most common symptoms observed during the first clinic assessment [56,57]. Moreover, patients often develop overlapping symptoms of different FTD subtypes as the disease progresses [58].

1.9.2 Initial Symptoms in Symptomatic Genetic FTD

Assessing the initial symptoms based on genetic mutation offers a unique opportunity to examine the behavioural, cognitive and neuropsychological disease-related changes that are

associated with a *known* underlying pathology. Studies assessing symptomatic carriers of *C9orf72* or *GRN* have found that behavioural/personality changes are a common initial complaint (C9orf72: [58,59]; GRN: [57,60]). As well, problems with memory and language may also occur (C9orf72: [61,62]; GRN: [57,63]). In carriers of *MAPT*, behavioural symptoms are reported to be the most common symptom [64-67], with memory and language impairments occurring at a lower frequency [64,67]. Currently, no study has evaluated and systematically compared the initial symptoms across the three main FTD-causing genetic mutations in a large cohort of patients. This knowledge will be instrumental in determining which biomarkers are most sensitive to each genotype, and for the design of clinical trials targeting preclinical mutation carriers and conversion to the symptomatic state.

1.9.3 Initial Symptoms in Asymptomatic Genetic FTD

It is currently unclear whether other neuropsychiatric, behavioural or cognitive changes may occur in FTD mutation carriers in the preclinical period, before the first classic symptoms of FTD are clearly present. Although studies have evaluated cognitive and neuropsychiatric alterations during the preclinical period [68-72], the occurrence of these symptoms across the three main mutation groups during the preclinical period has not been established. Moreover, other symptoms prevalent in FTD including disinhibition, apathy, social inappropriateness and altered food preferences have not been thoroughly explored in preclinical mutation carriers. Assessing the occurrence of FTD-related symptoms during the preclinical disease stage will indicate whether related measurements may be utilized to inform the initiation of treatments.

Overall, identifying gene-specific patterns of symptom endorsement during the preclinical period can inform outcome measures to assess the effectiveness of symptomatic

or disease-modifying treatments targeting different pathologies. Additionally, this knowledge can inform the selection of outcome measures in basket-design trials where the targeted therapy is assessed across different mutations with the same underlying pathology. Furthermore, evaluating the endorsement of FTD-related symptoms during the preclinical period across multiple domains of functioning can help determine whether specific symptom occurrence is related to the underlying pathology of FTD, a consequence of normal aging or due to the stress/burden of having a family member with FTD.

1.10 Brain Volumetric Changes in FTD

1.10.1 Grey Matter Atrophy Patterns in FTD

Frontal and/or temporal atrophy are the classic neuroimaging features in FTD. Early changes are evident within the orbitofrontal cortex, anterior cingulate cortex, superior temporal gyrus, medial frontal gyrus, insula, hippocampus, ventral striatum and thalamus [73,74]. Over time, atrophy progresses within the frontal, temporal and parietal lobes, specifically within the temporal lobes (bilaterally), left inferior frontal gyri, posterior cingulate (bilaterally), and right parietal lobe [75,76]. Despite the level of heterogeneity in imaging findings, there is a general consensus in the regional pattern of atrophy in each of the clinical syndromes. The bvFTD syndrome is characterized by volume loss in the frontal and temporal lobes, particularly in the prefrontal cortex, anterior temporal lobes, insula, anterior cingulate, striatum and thalamus. Atrophy in sv-PPA is predominant within the left temporal lobe, including the inferior temporal and fusiform gyri, temporal pole and the parahippocampal and entorhinal cortex. As well, left temporal lobe involvement is also found in nfvPPA, though mainly involves the inferior frontal gyrus, dorsolateral PFC, superior temporal gyrus and insula [77].

1.10.1.1 Grey Matter Atrophy Patterns by Gene

Distinct associations have been found between regional brain atrophy and the underlying pathogenic mutation. Relative to *MAPT* and *C9orf72* carriers, *GRN* mutation carriers demonstrate a faster rate of whole brain atrophy and asymmetry, with greater left versus right side involvement [78-80]. As well, in *GRN* carriers, grey matter loss is predominantly in the inferior and posterior temporal lobes, parietal lobes, posterior cingulate and precuneus [80-82]. Carriers of the *C9orf72* expansion show more wide-spread atrophy with involvement in the frontal lobes (orbitofrontal, medial prefrontal and dorsolateral PFC), temporal parietal, occipital lobes and cerebellum [81,83]. In *MAPT* carriers, grey matter loss is predominant in the anterior and medial temporal lobes [81-83]. Overall, there appears to be some overlapping yet distinctive patterns of atrophy across the main genetic forms of FTD.

1.10.1.2 Preclinical Grey Matter Changes.

Studies investigating asymptomatic genetic mutation carriers have found subtle brain volume alterations occurring prior to clinical onset. Specifically, as a group, preclinical mutation carriers exhibit significant grey matter loss relative to mutation non-carriers [84-86]. Furthermore, grey matter volume changes have been found to emerge 2 years prior to expected disease onset [68]. Within each genetic mutation cohort, the cortical atrophy in preclinical participants is similar to the expected pattern found in symptomatic patients [80,84,86-88]. Overall, the pathological process of FTD that results in atrophy begins during the preclinical period in genetic mutation carriers, prior to the onset of clinical symptoms.

1.10.2 Ventricular Volume in FTD

In addition to grey matter volume, one emerging marker of atrophy in neurodegenerative disease is the measure of ventricular volume. As ventricular volume expansion is consistently seen across the heterogenous clinical and genetic syndromes, ventricular volume may serve as a single measure to detect changes in FTD. Specifically, the contrast in intensity between the cerebrospinal fluid and surrounding tissue makes the ventricles an ideal region for automatic segmentation procedures [89], highlighting the efficiency as a potential neuroimaging tool. Furthermore, the position of the ventricles makes them less susceptible to distortions due to gradient non-linearity [89], and inhomogeneity artifacts compared to whole brain volume [90], supporting reliability of this method across clinical centres and scanners.

Across the FTD subtypes and genetic mutations, ventricular expansion is evident across the heterogenous clinical and genetic subtypes of FTD [56,79,91-95]. Only one study to date has examined ventricular expansion in preclinical mutation carriers and reported stable volumes over a 6-month follow-up period [96]; however, this study only assessed a single mutation group (*C9orf72*) and included a small sample (n=7).

Importantly, aside from Floeter, Bageac, Danielian, Braun, Traynor and Kwan [96], no study has assessed the sensitivity of ventricular volume measurements in preclinical mutation carriers in FTD. Identifying ventricular volume changes in genetically at-risk individuals could predict when the pathophysiology can be detected prior to disease onset. This knowledge may inform the timing of future therapeutic interventions and be used as outcome markers to assess treatment efficacy.

1.11 Functional Neural Changes in Symptomatic Frontotemporal Dementia

1.11.1 Behaviour Flexibility in FTD

Despite facing negative social, legal and physical consequences, patients with FTD exhibit difficulty modifying their behaviour. For example, patients with disinhibited symptoms continue to engage in inappropriate social behaviour (e.g. aggression, over-familiarity with strangers), exhibit a loss of manners (e.g. making insensitive comments, lack of social etiquette), or continue to overspend or gamble despite severe debt accumulation [40,97]. Pathophysiologic changes and atrophy in the frontal and temporal lobes have been found to be associated with these prevalent disinhibited behaviours. In patients, reduced cerebral blood flow within the OFC, bilateral inferior frontal gyrus (BA 47), left anterior cingulate gyrus (BA 32) and right caudate nucleus and left insula (BA 13), have been associated with greater engagement of antisocial behaviours [98]. Additionally, atrophy in the orbital and inferior frontal cortex, insula and right middle temporal regions has been associated with caregiver-reported disinhibited behaviours [99]. As disinhibition is one of the earliest symptoms to emerge in patients with FTD [40], and poses a great stress to caregivers [99], identifying effective treatments for these behaviours is essential. Furthermore, given the existing challenges in clinical trial design due to the relatively low base-rates of FTD and the diagnostic challenges, leading to recruitment challenges, the heterogeneity of clinical symptoms , and the time-intensive and costly nature of conducting trials, it is critical to identify markers that can guide treatment selection and be used as efficient outcome measures for clinical trials (i.e. proof of concept or challenge study) [6].

1.11.2 Reversal Learning

Reversal learning is a measure of adaptive behavioural flexibility that assesses the ability to alter behaviour when reinforcement contingencies change [100]. Performance on reversal learning task is associated with the degree of disinhibited and socially inappropriate behaviours exhibited by patients with frontal lobe lesions [100]. Through trial and error, participants learn stimulus-reward contingencies (*acquisition phase*), selecting stimuli associated with reward and avoiding stimuli associated with punishment. During the *reversal phase,* the reinforcement contingencies change, such that the stimuli that were previously associated with punishment are now associated with a reward, and those initially associated with punishment are now rewarded. The underlying neural regions and neurotransmitters mediating successful reversal learning are well-characterized in healthy populations. In conjunction with the extent literature, delineating the underlying neural deficits associated with reversal learning in FTD can determine the mechanisms mediating the associated behavioural deficits, and can inform potential targets for treatment interventions.

1.11.2.1 Neural Correlates of Reversal Learning

Based on fMRI and lesion studies in non-human primates and humans, specific neural regions have been found to be involved and/or make critical contributions to successful reversal learning including the orbital frontal cortex/ventromedial prefrontal cortex (OFC/vmPFC), ventrolateral PFC (vlPFC), dorsolateral PFC (dlPFC), and regions of the striatum.

1.11.2.2 OFC/vmPFC

Patients with OFC damage report awareness of the reversed feedback contingencies but are unable to adjust their behaviours accordingly [100,101]. It has been suggested that the OFC/vmPFC is critical when adjustments of behaviour are prompted following changes in reward contingency [100-104].

fMRI studies demonstrate that during the omission of an expected reward, the OFC/vmPFC demonstrates a decrease in signal from baseline (negative prediction error), whereas the presentation of an unexpected reward results in an increase in activity (positive prediction error) [103]. Following this, it has been suggested that the OFC/vmPFC encodes the reward values of objects/responses, as well as violations in this encoding through a prediction error which signals the discrepancy between the expected and actual reward [103]. In line with a prediction error formulation, the OFC/vmPFC has been found to be *less* active during reversal errors relative to rewarded correct responses [105,106].

1.11.2.3 Lateral PFC

The dlPFC has been found to be integral for aspects of cognition that are important during successful reversal learning including attention shifting [107,108], and learning reward and punishment information for decision making [102,108]. Lesion studies have reported mixed findings regarding the necessity of the lateral frontal cortex in reversal learning. Studies have reported that lesions to the dlPFC does not impact reversal learning [102,107]. However, during more complex reversal learning tasks that include probabilistic feedback, the dlPFC seems to be critical for successful performance [101,109].

Functional neuroimaging studies have implicated the role of the lateral PFC during reversal learning. Specifically, the dlPFC has been found to respond to decision conflict during

reversal of a reward [105,110,111], and when differentiating between two reward options that are similar in value [110]. As the dlPFC responds to decision conflict, it has been argued that this region augments the representation of relevant stimuli and reinforcement information to guide subsequent behaviours [105,112]. The vlPFC has been shown to be engaged when suboptimal responses have been made (a reversal error), and thus, an alternative behaviour is warranted [105,110,111]. Specifically, Cools, et al. [113] demonstrated that the vlPFC responded to the last reversal error preceding a successful reversal in behaviour. Thus, it has been proposed that this region is involved in selecting appropriate behaviours/motor responses. In fact, Budhani, Marsh, Pine and Blair [105] suggested that following a conflict (e.g. reversal error), the dlFC representing the stimulus features of the object within the vlPFC which then control motor responding to alter subsequent behaviour.

1.11.2.4 Striatum

Lesion studies also propose a fronto-striatal circuit that is critical for processing reward and punishment and mediating behaviours during reversal learning [114,115]. For example, Divac, et al. [116] demonstrated that monkeys with lesions to the ventrolateral region of the head of the caudate, a region which receives connections from the OFC, experience deficits in the reversal learning component of the Wisconsin General Test. Furthermore, excitotoxic lesions to the striatum *or* the OFC impaired the ability to reverse stimulus-reward contingencies [115]. Overall, functional and lesion studies suggest that the striatum interacts with regions within the frontal cortex to mediate subsequent responding when a behavioural adjustment is warranted.

Functional neuroimaging studies have also implicated the involvement of the striatum in response to reversal errors [117-119]. In particular, the ventral striatum has been shown to be

engaged during the final reversal error prior to a behavioural change [113]. Other studies have reported the engagement of the caudate during punished reversal errors [105] and when new searches are initiated (e.g. during reversal or stimulus-set change) [120]. Dissociable regions of the striatum have been shown to be engaged in separate elements of stimulus-response learning. Specifically, the dorsal striatum has been shown to be engaged during response selection and is proposed to mediate decision making. In contrast, the ventral striatum has been found to be engaged during feedback and is proposed to underlie learning associations between stimuli and responses [121]. Likewise, O'Doherty [103] suggested the role of the ventral striatum in representing predicted future reward values, and the dorsal striatum in learning the specific actions that need to be performed to obtain the reward (stimulus-response associations). As well, it has been proposed that the caudate is involved in maintaining adaptive goal-directed behaviour by evaluating action-outcome contingencies [122]. During reversal learning tasks, it has been proposed that the caudate interacts with the vlPFC during instances of response conflict (i.e. receiving negative feedback), to augment motor responses [105,118]. In line with this model, Mitchell, Rhodes, Pine and Blair [118] demonstrated increased activity within the vlPFC and caudate during instances of conflict (e.g. reversal errors, non-reversal errors and correct reversal responses).

1.11.3 Neurotransmitters involved in Reversal Learning

Pharmacological studies have revealed a differential contribution of monoamine neurotransmitters during reversal learning. Serotonin depletion in the marmoset PFC results in perseverative responding to previously rewarded stimuli, suggesting a role for serotonin in behavioural flexibility [123]. This role of serotonin has been suggested to be specific to the OFC

as serotonergic depletion in the medial caudate does not impact reversal learning performance [124], or attentional set-shifting, which is mediated by the dlPFC [107,125].

Dopamine neurons encode prediction error signalling [126] which has been found to be mediated by the OFC, amygdala and ventral striatum [103]. Dopaminergic depletions of the marmoset OFC do not impact reversal learning performance [127]; however, depletion in the medial caudate leads to reversal deficits [124]. Studies in Parkinson's Disease (PD) have suggested that dopaminergic medication may overdose brain regions with relatively intact levels of dopamine including the ventromedial caudate, and replete dopamine-depleted regions including the dorsal striatum [128,129]. Consequently, patients with PD exhibit impaired reversal learning performance on dopaminergic medication relative to off medication [129,130]. As well, in healthy controls with presumably optimal baseline dopamine levels, administration of levodopa resulted in greater reversal errors relative to when participants were on placebo [131].

1.11.4 Reversal Learning in FTD

Consistent with the suggestion that patients with FTD exhibit deficits in flexibly modifying behaviour, the available evidence suggests that FTD is associated with reversal learning impairments [132-134]. Although patients adequately associate each stimulus with its reinforcement value, when the reward contingencies change, patients continued to select the previously rewarded stimulus, and thus make more reversal errors relative to controls [132,134]. One study has found an association between the number of reversal errors made and atrophy within the anterior cingulate cortex (BA 24) and the medial/lateral OFC (BA 11, 47) [133]. This suggests that the inability to suppress a previously rewarded behaviour in favour of an alternative behaviour may be associated with degeneration of the frontal cortex.

To date, no study has evaluated the functional neural correlates of reversal learning in FTD. Delineating the pathophysiology of impaired behavioural flexibility in FTD can inform whether reversal learning deficits are the result of impaired prediction error signalling during an unexpected reinforcement (vmPFC), impaired conflict processing (dmPFC), and/or implementing alternative motor responses (caudate/vlPFC). These findings may provide targets for future pharmacological or behavioural interventions mediating these underlying cognitive functions.

1.12 Thesis Objectives and Hypothesis

The **overall objective** of this thesis is to elucidate behavioural, structural and functional changes in FTD from the preclinical to the symptomatic disease stage. This goal was addressed using a multifaceted approach whereby three independent studies were conducted in patients with FTD and/or individuals who are genetically at-risk for developing FTD. The **central hypothesis** is that potential markers and outcome measures for clinical trial designs will be identified in preclinical and symptomatic individuals. Ultimately, the results of these studies will extend previous work on the potential candidate biomarkers sensitive to FTD to help diagnose patients earlier and more accurately, and characterize the functional processes that underlie symptoms of FTD to identify potential targets for treatments, and inform clinical trial designs. Additionally, these results may help delineate the natural progression of this disease from the preclinical, prodromal and affected stages and supplement our knowledge on the vulnerable brain networks that can be targets for future therapies.

Study 1: Early Symptoms in Symptomatic and Preclinical Genetic Frontotemporal Lobar Degeneration

Identification of the initial symptoms that represent the earliest functional manifestations of the pathophysiology of FTD are a critical outcome measure for future interventions aiming to prevent conversion to clinical FTD. The **first objective** of this study was to evaluate whether preclinical carriers of a pathogenic mutation demonstrate greater or unique symptoms relative to biologically related mutation non-carriers. The **second objective** was to evaluate whether the initial symptoms differed as a function of the specific genetic mutation during the preclinical and symptomatic period. Although some symptoms may be present in both familial mutation carriers and non-carriers due to shared environmental factors and stress/burden of having a family member with FTD, we **hypothesize** that as preclinical carriers approach the time of expected disease onset, they will endorse greater FTD-related symptoms relative to non-carriers. Additionally, consistent with the previous literature [83], we predict that specific symptom endorsement will differ across the genetic mutations within the preclinical and symptomatic mutation carriers.

Study 2: Ventricular Volume Expansion in Preclinical Genetic Frontotemporal Dementia

Some work has evaluated ventricular volume in patients with FTD and has found greater ventricular expansion in FTD relative to healthy controls [56,95]; however, no study has assessed ventricular volume expansion in preclinical mutation carriers in FTD. Ventricular volume measures are robust to scanner inhomogeneities and are amenable to robust automatic segmentation due to the intense contrast in intensity between the ventricles and surrounding tissue (Nestor et al, 2008). Investigating ventricular volume measurements during the preclinical

disease period will inform whether these measurements can be used to identify pathogenic changes prior to disease onset. The **objective** of the present study was to examine ventricular volume change over a one-year period in carriers of an FTD-causing mutation and non-carriers, to determine its potential utility as a biomarker of early preclinical disease. In line with previous research showing smaller cortical volumes during the preclinical period in mutation carriers [135], we **hypothesize** that mutation carriers (symptomatic and preclinical mutation carriers) will show greater ventricular expansion relative to non-carriers. Additionally, we predict that preclinical mutation carriers alone will show greater ventricular expansion relative to non-carriers.

Study 3: Neural Correlates of Reversal Learning in Frontotemporal Dementia

Disinhibition and poor behaviour flexibility is an early and debilitating symptom in FTD; thus, investigating the neural mechanisms mediating these symptoms is warranted. Specifically, assessing the underlying neural correlates can inform whether these behavioural deficits are the result of impaired prediction error signalling during an unexpected reinforcement (vmPFC), impaired conflict processing (dmPFC), and/or deficits in implementing alternative motor responses (caudate/vlPFC). Delineating the aberrant pathophysiology may inform potential pharmacologic or behavioural interventions. With this, the **objective** of this study to identify whether patients with FTD reveal abnormal neural deficits in an fMRI-reversal learning task. We **hypothesize** that patients with FTD will reveal abnormal activity in areas important in reversal learning including the mPFC (dorsal and ventral regions), and ventrolateral PFC, during the reversal learning task, especially during the reversal trials.

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Chapter 2: Early Symptoms in Symptomatic and Preclinical Genetic Frontotemporal Lobar Degeneration[1]

2.1 Introduction

Frontotemporal dementia (FTD) is a neurodegenerative disorder with approximately 40% of patients showing a strong family history, with mutations in the chromosome 9 open reading frame 72 (C9orf72), progranulin (GRN) or microtubule-associated protein tau (MAPT) genes each accounting for 5-10% of patients with FTD [1]. While therapies targeting the underlying pathology are in development [2], currently, no treatments are available to prevent or alter the course of disease progression.

Even during the early stages of disease, symptoms of FTD are quite impairing [3]; thus, treatments will likely need to be administered during the preclinical stage, before a patient meets the current international consensus criteria [4,5]. Consequently, there is a growing interest in identifying biomarkers and clinical endpoints that can best inform when to administer these interventions and how to track treatment efficacy. A major challenge in designing clinical trials and the designation of clinical endpoints is the heterogeneity of genetic FTD at the phenotypic [6], and pathological levels [7,8]. For instance, clinical symptoms in genetic FTD range from language disturbances [5] to behavioural and neuropsychiatric features [4], which occur at various frequencies and ages even within families, and have different neuroanatomic associations [9,10]. Furthermore, at present, it is not yet known whether or when symptoms associated with

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genetic FTD may occur during the prodromal period, and whether such symptoms may be specific to the later development of clinical FTD.

To inform clinical endpoint selection for future clinical trials in at-risk cohorts, the first objective of the current study was to evaluate the most frequent initial symptoms in patients with symptomatic genetic FTD due to C9orf72, GRN or MAPT mutations. The second objective was to evaluate whether preclinical mutation carriers demonstrate greater or different symptoms relative to biologically related non-carriers during the preclinical period.

2.2 Method

2.2.1 Participants

The current study used data from the Genetic Frontotemporal Dementia Initiative (GENFI) multicentre cohort study, which consists of research centres across Europe and Canada (http://genfi.org.uk/). This dataset is comprised of (1) known symptomatic carriers of a pathogenic mutation in the GRN or MAPT genes or with a pathogenic expansion in the C9orf72 gene (greater than 30 repeats) with clinical diagnoses based on the international consensus diagnostic criteria [4,5], and (2) first-degree biological family members of a known GRN, MAPT or C9orf72 mutation carrier who are at-risk for developing FTD and were not yet demonstrating evidence of progressive cognitive or behavioral symptoms (including both preclinical carriers and non-carriers). All eligible and interested participants were enrolled in the study. Importantly, the majority of at-risk family members in the GENFI study, and the local GENFI research teams, were not aware of their genetic status at the time of the assessments. After their baseline visit, participants were followed for up to five annual visits. All participants had an identified informant who completed clinical scales (see below). Participants with completed study

measures were included in the analysis; information on other demographic variables was complete for all participants in the study. The data was part of the GENFI data freeze 4 collected at 22 GENFI sites (2012-2018). Local ethics committees at each site approved the study and all participants provided written informed consent at enrollment.

2.2.2 Study Measures

GENFI Symptom List: The initial 37-symptom list was designed to include a variety of FTD-related symptoms based on standardized rating scales (Appendix A: method section 1.0, Table A.1, A.2 and result section 2.0). Informants of symptomatic patients (typically a spouse or sibling) described the initial symptom and trained research coordinators selected the corresponding symptom from the list. For at-risk family members, clinicians completed the GENFI symptom list with the at-risk family member and their study informant, and evaluated the presence of each symptom using a 5-point Likert scale (0=absent, 0.5= questionable/very mild, 1=mild, 2=moderate, 3=severe). Symptom ratings of questionable/very mild, mild, moderate, severe were coded as symptom endorsement and absent coded as symptom absent.

2.2.2.1 Cambridge Behavioural Inventory Questionnaire-Revised (CBI-R)

Informants of at-risk family members completed the CBI-R [11]. This questionnaire was used to evaluate the at-risk groups' symptoms within the past 4 weeks. Each question is evaluated on a 5-point scale, where higher scores indicate greater symptom endorsement and severity. Symptom domains included memory and orientation, everyday skills, self-care, abnormal behaviour, mood, beliefs, eating habits, sleep, stereotypic and motor behaviours and motivation. Each domain includes 2 to 8 sub-items.

Years from expected onset was used to determine whether participants who were closer to the age of anticipated clinical onset endorsed greater symptoms. Years from expected onset (YEO) was calculated by subtracting the mean age of clinical onset within the family from the participant's current age [10,12]. Negative values denote that the participant is at an age prior to expected clinical onset; positive values indicate that the participant is at an age after expected clinical onset.

2.2.3 Statistical Analysis

GENFI Symptom List: Descriptive statistics were used to illustrate the most frequent symptoms endorsed at participants' initial visits. Differences amongst the three genetic groups in the frequency of the most prevalent sub-symptoms were examined using Chi-squared test or Fisher's exact test for the symptomatic patients and at-risk individuals, and separately comparing preclinical mutation carriers and non-carriers for each gene mutation. Mixed models were not used to account for potential clustering effects of family membership and site, due to the low symptom endorsement (creating small samples) by patients and at-risk family members.

For symptomatic and at-risk family members, a composite index was created for each gene based on three most frequently endorsed initial symptoms for each of the symptomatic genetic groups (C9orf72 & MAPT: disinhibition, apathy, memory; GRN: apathy, articulation, fluency). For each composite, participants attained a score of 1 if they endorsed at least one symptom within each composite (0=no symptoms endorsed, 1= at least one symptom endorsed). Note only the predominant initial symptom was recorded in the GENFI intake for affected participants. To evaluate the effectiveness of this composite to differentiate between mutation

carriers and non-carriers, sensitivity and specificity values were computed (https://www.medcalc.org/calc/diagnostic_test.php).

To evaluate changes in symptom endorsement over time in at-risk family members who had at least one follow-up visit, a difference score was calculated by subtracting symptom endorsement at the final visit from symptom endorsement at the first visit (0=not endorsed, 1=symptom endorsed). This resulted in three categories for each symptom: decrease in symptom endorsement over time (score of -1), no change in symptom endorsement over time (score of 0), increase in symptom endorsement over time (score of 1). Calculating change scores enabled all participants to be included in the analysis, regardless of the number of follow-up visits. Chi-squared tests/Fisher's Exact tests were completed to assess group differences.

To evaluate whether the initial symptoms were similar amongst patients from the same family, a congruency score was calculated as the number of pairwise comparisons in which family members shared an initial symptom, divided by the total number of possible pairwise comparisons. A congruency score was also calculated to evaluate the congruency of initial predominant symptoms for specific GRN and MAPT mutations.

2.2.3.1 Cambridge Behavioural Inventory Questionnaire-Revised

A generalized linear mixed model with a Laplace likelihood approximation function was used to examine differences in the total CBI-R scores between preclinical mutation carriers vs. non-mutation carriers at the initial GENFI visit as a function of years from expected clinical onset. This analysis accounted for potential clustering effects based on family membership. Plots of the CBI-R total scores suggested a Poisson distribution; however, due to overdispersion as indicated through the Pearson Chi-Square/DF (4.62), a negative binomial distribution with a log

link function was used. For the total score, no participant had studentized residuals greater than +/- 3.. Predictor variables included random effects [family membership] and fixed effects [genetic status (preclinical vs. non-carriers), years from expected onset, and an interaction between genetic status and years from expected onset]. Examination of the residuals suggested the use of weights to account for the within-family correlation in the model. Given the variability in contribution of family membership to predicting age of onset by mutation group [10], a confirmatory analysis was conducted substituting years from expected onset with the participant's age. Of note, as age was highly correlated with years from expected onset ($r=0.84$, $p<0.001$), participant's age could not be included in the model due to multicollinearity. However, when age was substituted for estimated years from expected onset, the pattern of results was similar (Table A.3).

Change scores (symptom score at final visit – score at first visit)/ time interval) were calculated to compare longitudinal data. Participants with studentized residuals greater than +/- 3 were removed (Table A.4), and a linear mixed model was used (see Appendix A, method section 3.0 on the description of the model formation). Predictor variables included random effects [family membership] and fixed effects [genetic status (preclinical vs. non-carriers), years from expected onset or participant's age, CBI total score at baseline, and an interaction between genetic status and years from expected onset]. A confirmatory analysis was run substituting participant's age at baseline for the years from expected onset (Table A.3). As differences between the preclinical and non-carriers in the total CBI scores may be obscured by opposed group differences in the sub-scale scores, we also examined group differences at baseline and longitudinally for each of the sub-scales by using the model developed for the total score. For these models, the same parameters were used with one exception: the sub-scale score at baseline

was used as a fixed effect instead of the CBI total score at baseline. For both the baseline and change score analysis, the potential influence of specific FTD-causing mutations was examined by assessing the impact of genetic mutation type as the grouping variable (C9orF72, GRN, MAPT, mutation non-carriers), and post-hoc comparisons were conducted between each genetic group and non-carriers. For brevity, the results from the models with the genetic mutation group are reported in the manuscript.

2.3 Results

2.3.1 Participants

185 patients diagnosed with FTD (C9orf72 n=87, GRN n=65, MAPT n=33) were included in the analysis. Additionally, two groups of at-risk family members completed the GENFI symptom list and CBI-R scales: 637 at-risk family members (317 preclinical mutation carriers, 320 mutation non-carriers) and 588 at risk individuals (294 preclinical carriers, 294 non-carriers) completed the GENFI symptom list and CBI-R scales, respectively (Table 2.1).

2.3.2 Predominant Initial Symptoms in Symptomatic Patients

Across the entire cohort the most frequently endorsed initial symptoms were apathy (23%), disinhibition (18%), memory impairments (12%) decreased fluency (8%) and impaired articulation (5%; Figure 2.1, Table A.5). When the most frequent initial symptoms were compared amongst the mutation groups, patients with MAPT mutations presented with disinhibition more frequently relative to C9orf72 and GRN carriers, and displayed memory impairments more frequently than GRN carriers. GRN carriers exhibited impaired articulation

and decreased fluency more often than C9orf72 and MAPT carriers. No group differences were observed for apathy.

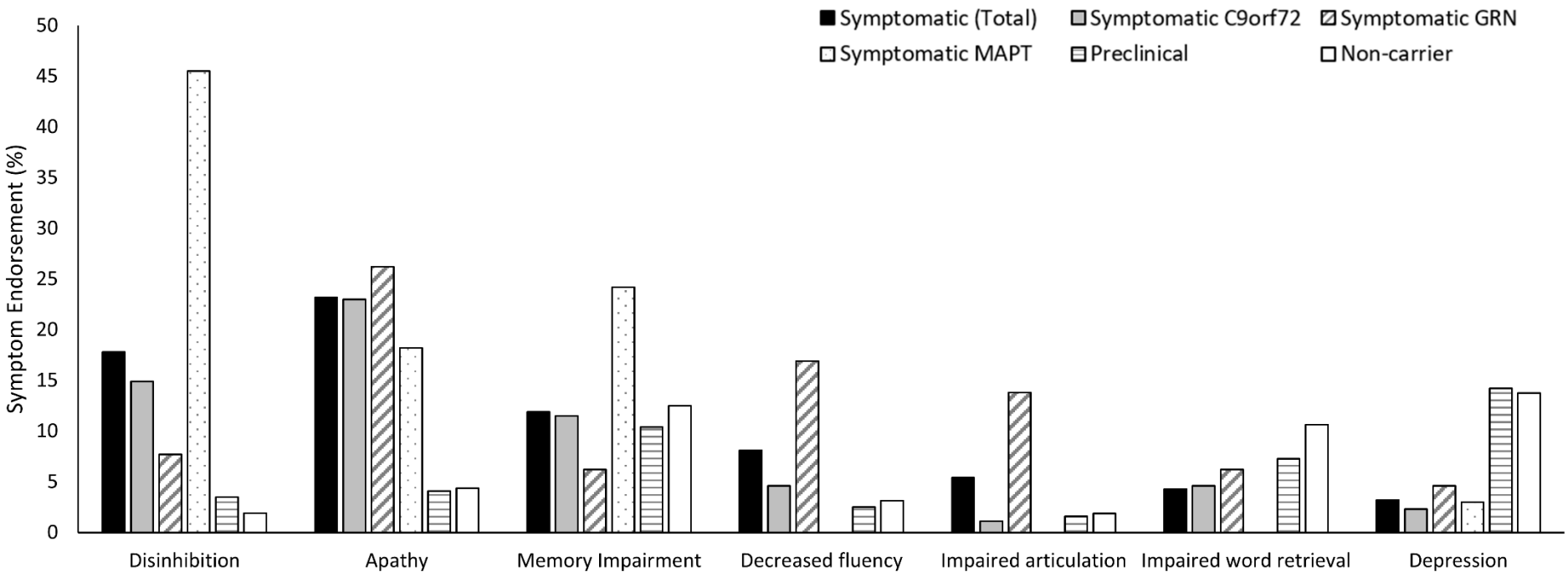
Table 2.1: Demographics table for symptomatic and at-risk family members

	Symptomatic Patients					At-risk Family Members					
	Total	***C9orf72***	***GRN***	***MAPT***	**Contrasts**	**Preclinical[&]**	**Non-carrier[&]**	**Contrasts[&]**	**Preclinical[^]**	**Non-carrier[^]**	**Contrasts[^]**
N	185	87	65	33		317	320		294	294	
Handedness					p=0.02[*#]			p=0.16[*#]			p=0.14[*#]
Right	174	80	65	29		282	298		275	262	
Left	9	5	0	4		31	20		17	28	
Ambidextrous	2	2	0	0		4	2		2	4	
Sex					X^2=6.2, p=0.045			X^2=0.90, p=0.34			X^2=0.86, p=0.35
Male	108	57	30	21		123	136		112	123	
Female	77	30	35	12		194	184		182	171	
Genotype								X^2=0.21, p=0.90			X^2=0.58, p=0.75
C9orf72						117	115		104	103	
GRN						144	144		138	132	
MAPT						56	61		52	59	
Maximum number of visits											
1						121	118		124	122	
2						80	98		80	95	
3						72	58		60	38	
4						30	27		22	23	
5						10	15		7	16	
6						4	4		1	0	
Diagnosis											
bvFTD		62	33	31							
PPA		4	28	0							
FTD-ALS		9	0	0							
ALS		6	0	0							
PSP		1	0	0							
CBS		0	2	1							
AD		0	1	0							
Dementia- NOS		3	1	1							
Other		2	0	0							
Time interval for change score (SD)						2.6 (1.4) [n=196]	2.5 (1.5) [n=202]	t(394.7) = -0.6, p=0.54	2.5 (1.3) [n=170]	2.4 (1.5) [n=172]	t(340)= -0.7, p=0.49
Age (SD)	62.3 (8.5)	63.7 (8.3)	63.5 (6.9)	56.2 (9.5)	F(2,184)=11.5, p<0.001[#] C9> MAPT GRN > MAPT	44.0 (11.8)	46.3 (14.0)	t(619)=2.3, p=0.03	44.0 (11.9)	46.7 (14.1)	t(570.1)=2.6, p=0.01
Age at onset (SD)	58.1 (8.8)	58.8 (9.0)	60.6 (7.2)	51.1 (7.7)	F(2,184)=11.5, p<0.001[#] C9>MAPT GRN >MAPT						

Education, Yrs, (SD)	12.2 (4.0)	12.6 (4.0)	11.2 (4.0)	13.2 (3.6)	F(2,184)=3.5, p=0.03[#] MAPT> GRN (p=0.065)	14.3 (3.3)	13.9 (3.6)	t(635)= -1.5, p=0.13	14.3 (3.3)	13.9 (3.6)	t(586)= -1.58, p=0.1
Years from expected disease onset (SD)[**]						-14.4 (11.8)	-13.2 (14.1)	t(618.5) = 1.17, p=0.24	-14.5 (12.0)	-12.9 (14.2)	t(569.3)= 1.51, p=0.13

- Chi-squared (X^2), Fisher's Exact tests (if expected cell count was less than 5), independent sample t-tests or one-way analysis of variance were used to discern group differences for relevant variables
- # Bonferroni correction applied
- [&]At-risk participants from 248 families. Participants completed the GENFI symptom list
- ^At-risk participants from 228 families. Participants completed the CBI questionnaire
- *#Fisher's Exact Test was used
- **Years from expected onset was calculated by subtracting the participant's age at the time of participation from the mean age of disease onset within the family

Figure 2.1. Symptom endorsement in symptomatic patients and at-risk family members



Percentage of patients and at-risk individuals that endorsed symptoms identified as the most frequent symptoms in symptomatic patients.

2.3.3 Symptom Congruency

Fourteen families had at least two related patients in the study cohort; amongst these families, the average percentage congruency for first symptom similarity was 19% (Table A.6). Five families with a MAPT mutation and 7 families with a GRN mutation had at least two related symptomatic patients in the study cohort and the specific genotype was known. Of the specific genotypes, the average congruency score was 33% for MAPT and 20% for GRN mutations (Table A.7).

2.3.4 Symptom Endorsement in at-risk Family Members (GENFI symptom list)

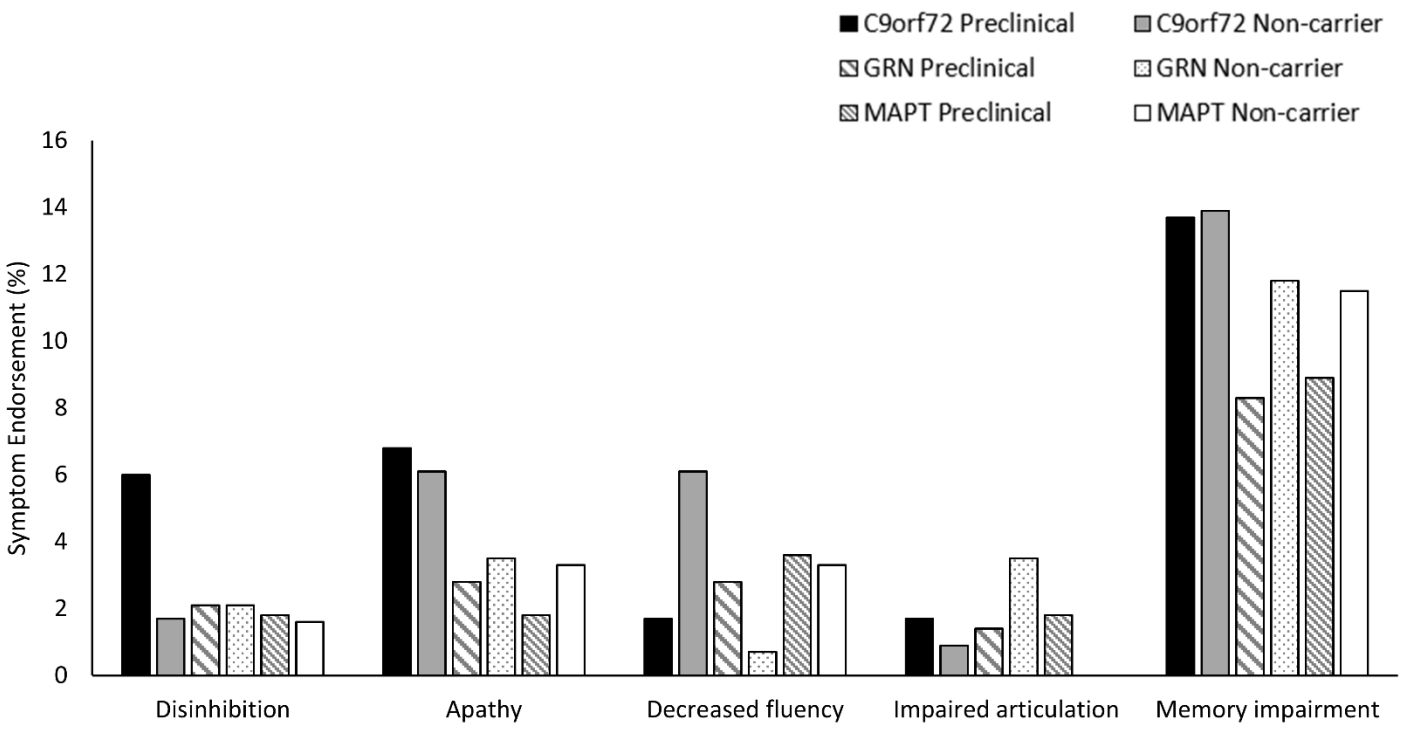
There were no significant differences between at-risk individuals (preclinical C9orf72, GRN, MAPT vs. non-carriers) or between preclinical genetic groups in the proportion of participants who endorsed the initial symptoms most commonly reported in affected patients (i.e. apathy, disinhibition, decreased fluency, impaired articulation and memory impairments) (Figure 2.2 & Table A.5, A.8). Overall, at-risk genetic groups (preclinical C9orf72, GRN, MAPT vs. non-carriers) showed a similar pattern of symptom endorsement over time, with a very low proportion of participants reporting changes in the most common initial symptoms (Table A.9).

3.3.5 Composite Scores

The sensitivity and specificity values indicate the composite indices differentiate between symptomatic FTD and mutation non-carriers for each of the gene groups with sensitivities from 94% to 97% and specificities of 80%. For at-risk family members, the composite indices showed low sensitivity (8-33%), with medium specificity (76-91%) to differentiate between preclinical

mutation carriers from non-carriers beginning from -5, -2 and 0 years to expected age of onset (Table A.10, A.11).

Figure 2.2. Baseline symptom endorsement by genotype in at-risk family members



Percentage of preclinical and non-mutation carriers that endorse each of the sub-symptoms identified as the most frequent symptom in symptomatic patients

2.3.6 Symptom Endorsement & Severity in at-risk Family Members (CBI-R questionnaire)

CBI-R scores at baseline: As participants approached the anticipated time of onset there was a significant increase in the reported total symptom score, memory and orientation, sleep, motivation, eating habits, and stereotypic and motor behaviours scores. When adjusting for expected years to onset and relative to non-carriers, post-hoc contrasts showed that MAPT

carriers experienced greater mood, sleep, and motivation symptoms; C9orf72 carriers endorsed greater abnormal behaviour and stereotypic & motor symptoms; and GRN carriers had lower mood scores (Table 2.2; Figure 2.3).

Longitudinal CBI scores: Improved symptoms over time (negative change scores) were associated with greater symptom scores at baseline when adjusted for expected years to onset and carrier status across all participants. There were also significant associations between expected years to onset and memory and orientation scores, stereotypic and motor behaviours, but also for eating habits (Table 2.2). Within the sub-scales, GRN and C9orf72 preclinical carriers demonstrated worse everyday skills over time relative to mutation non-carriers, but only the GRN carriers' scores met statistical significance (Figure 2.4).

2.4 Discussion

As the first study to compare initial symptoms in symptomatic and at-risk patients with genetic FTD across the three main genetic mutations *MAPT*, *C9orf72* and *GRN*, our findings demonstrate the overlap and differences in the presence and frequencies of specific FTD-related symptoms. We also report the first longitudinal differences between preclinical mutation carriers in comparison to familial non-carriers in the endorsement of symptoms prior to diagnosis. Important to the interpretation of symptom reports and design of clinical trials, we found that preclinical *MAPT* and *C9orf72* mutation carriers endorsed greater symptoms at the initial assessment (approximately 14 years prior to anticipated age of onset), and over time *GRN* and *C9orf72* mutation carriers exhibited poorer everyday skills. The direct comparison of symptoms among mutation groups may be important in the consideration of basket-design clinical trials where, for example, patients with TDP-43 pathology arising from different mutations (*C9orf72* & *GRN*) may be grouped together.

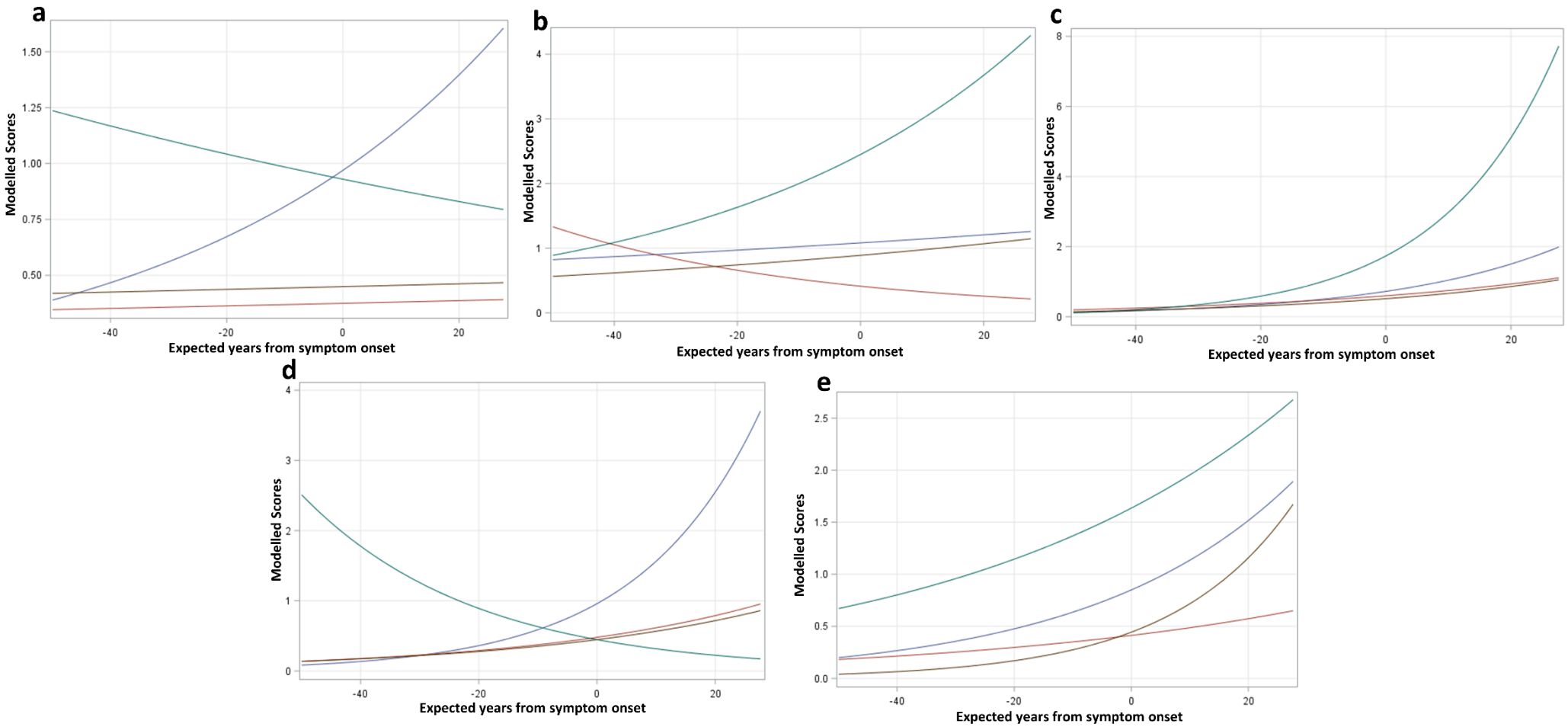
Table 2.2: CBI-R total and sub-scale scores at baseline and over time for at-risk family members by genetic group (no outliers included)

	Baseline[#]			Change Score		
	N	**Estimate (95% CI)**	***p*-value**	**N**	**Estimate (95% CI)**	***p*-value**
Total Score	588			336		
C9orf72	104	1.34 (0.78, 2.31)	0.29		0.28 (-1.42, 1.97)	0.75
GRN	138	0.95 (0.52, 1.73)	0.86		0.38 (-0.8, 1.56)	0.53
MAPT	52	1.96 (0.88, 4.38)	0.1		0.39 (-1.37, 2.15)	0.66
YEO		1.02 (1, 1.03)	0.02		0.03 (-0.01, 0.07)	0.11
Baseline score		-	-		-0.15 (-0.21, -0.1)	<.0001
***C9orf72**YEO**		1 (0.98, 1.03)	0.8		0.01 (-0.08, 0.11)	0.78
***GRN**YEO**		1 (0.97, 1.03)	0.87		-0.02 (-0.08, 0.05)	0.63
***MAPT**YEO**		1 (0.96, 1.05)	0.85		-0.01 (-0.12, 0.1)	0.86
Memory and Orientation	588			334		
C9orf72	104	0.88 (0.51, 1.52)	0.65	49	-0.02 (-0.41, 0.37)	0.92
GRN	138	1.03 (0.56, 1.89)	0.92	85	-0.03 (-0.3, 0.25)	0.85
MAPT	52	0.89 (0.39, 2.03)	0.78	33	-0.01 (-0.42, 0.41)	0.98
YEO		1.03 (1.01, 1.04)	0.001		0.01 (0.002, 0.02)	0.02
Baseline score		-	-		-0.18 (-0.23, -0.13)	<.0001
***C9orf72**YEO**		0.98 (0.96, 1.01)	0.29		-0.003 (-0.02, 0.02)	0.74
***GRN**YEO**		1.01 (0.98, 1.04)	0.47		-0.002 (-0.02, 0.01)	0.78
***MAPT**YEO**		0.99 (0.95, 1.03)	0.59		0.0003 (-0.02, 0.03)	0.98
Everyday Skills	588			335		
C9orf72	104	0.77 (0.09, 6.56)	0.81	50	0.07 (-0.01, 0.14)	0.09
GRN	138	0.71 (0.1, 4.92)	0.72	85	0.11 (0.05, 0.16)	0.0001
MAPT	52	1.08 (0.05, 22.27)	0.96	32	0.03 (-0.06, 0.11)	0.53
YEO		1.03 (0.97, 1.09)	0.34		0.001 (0, 0)	0.57
Baseline score		-	-		-0.5 (-0.55, -0.45)	<.0001
***C9orf72**YEO**		1 (0.89, 1.13)	0.96		0.003 (0, 0.01)	0.21
***GRN**YEO**		1.05 (0.93, 1.2)	0.42		0.003 (0, 0.01)	0.07
***MAPT**YEO**		0.96 (0.82, 1.11)	0.57		0.0002 (0, 0.01)	0.95
Abnormal Behaviour	588			334		
C9orf72	104	2.16 (1.09, 4.26)	0.03	48	-0.02 (-0.3, 0.25)	0.86
GRN	138	0.83 (0.36, 1.91)	0.67[+]	86	-0.03 (-0.22, 0.15)	0.73
MAPT	52	2.07 (0.8, 5.38)	0.14	33	-0.02 (-0.3, 0.26)	0.89
YEO		1 (0.98, 1.02)	0.9		0.004 (0, 0.01)	0.19
Baseline score		-	-		-0.23 (-0.28, -0.18)	<.0001
***C9orf72**YEO**		1.02 (0.98, 1.06)	0.37		-0.006 (-0.02, 0.01)	0.47
***GRN**YEO**		1 (0.96, 1.04)	0.99		-0.007 (-0.02, 0)	0.23
***MAPT**YEO**		0.99 (0.95, 1.04)	0.77		-0.0033 (-0.02, 0.01)	0.71
Mood	587			334		
C9orf72	104	1.22 (0.7, 2.12)	0.49	49	-0.07 (-0.47, 0.34)	0.75
GRN	137	0.46 (0.23, 0.93)	0.03	84	0.18 (-0.11, 0.47)	0.2
MAPT	52	2.75 (1.29, 5.89)	0.01	33	0.38 (-0.05, 0.81)	0.08
YEO		1.01 (0.99, 1.03)	0.26		-0.002 (-0.01, 0.01)	0.7
Baseline score		-	-		-0.23 (-0.28, -0.18)	<.0001
***C9orf72**YEO**		1 (0.97, 1.03)	0.80		-0.018 (-0.04, 0)	0.11
***GRN**YEO**		0.97 (0.94, 1)	0.05		-0.003 (-0.02, 0.01)	0.73
***MAPT**YEO**		1.01 (0.97, 1.05)	0.58		0.0031 (-0.02, 0.03)	0.81
Beliefs				340		
C9orf72				49	-0.004 (-0.02, 0.01)	0.56
GRN				86	-0.01 (-0.02, 0.0014)	0.097
MAPT				33	-0.01 (-0.02, 0.01)	0.46
YEO					0.00007 (-0.0002, 0.0004)	0.62
Baseline score					-0.38 (-0.41, -0.34)	<.0001
***C9orf72**YEO**					-0.00017 (-0.0009, 0.0005)	0.64
***GRN**YEO**					-0.00017 (-0.0007, 0.0004)	0.52
***MAPT**YEO**					-0.0001 (-0.0009, 0.0007)	0.86

Eating habits	588			335		
C9orf72	104	0.61 (0.16, 2.32)	0.46	49	-0.02 (-0.2, 0.16)	0.83
GRN	138	1.57 (0.46, 5.39)	0.47	86	0 (-0.13, 0.1247)	0.99
MAPT	52	0.68 (0.1, 4.82)	0.70	32	0.1 (-0.09, 0.29)	0.29
YEO		1.05 (1.01, 1.09)	0.01		0.0041 (0.0001, 0.008)	0.04
Baseline score		-	-		-0.35 (-0.39, -0.31)	<.0001
***C9orf72**YEO**		0.96 (0.89, 1.03)	0.25		-0.006 (-0.02, 0.005)	0.28
***GRN**YEO**		1 (0.94, 1.07)	0.91		-0.00002 (-0.007, 0.007)	0.996
***MAPT**YEO**		0.95 (0.87, 1.05)	0.35		0.003 (-0.008, 0.01)	0.6
Sleep	588			334		
C9orf72	104	1.4 (0.75, 2.64)	0.29	49	-0.13 (-0.39, 0.13)	0.33
GRN	138	1.16 (0.56, 2.39)	0.68	86	0.05 (-0.14, 0.23)	0.62
MAPT	52	3.37 (1.46, 7.74)	0.004	32	0.02 (-0.26, 0.3)	0.89
YEO		1.03 (1.01, 1.05)	0.01		-0.0009 (-0.007, 0.005)	0.76
Baseline score		-	-		-0.28 (-0.33, -0.22)	<.0001
***C9orf72**YEO**		1.01 (0.97, 1.05)	0.56		-0.008 (-0.02, 0.006)	0.25
***GRN**YEO**		1 (0.96, 1.04)	0.86		0.003 (-0.008, 0.01)	0.63
***MAPT**YEO**		1.03 (0.98, 1.08)	0.26		-0.005 (-0.02, 0.01)	0.54
Stereotypic and motor behaviours	588			335		
C9orf72	104	2.15 (1.05, 4.39)	0.04[&]	49	-0.12 (-0.42, 0.18)	0.44
GRN	138	1.07 (0.46, 2.52)	0.87	86	0.08 (-0.13, 0.28)	0.47
MAPT	52	1 (0.31, 3.23)	0.999	32	0.002 (-0.31, 0.32)	0.99
YEO		1.02 (1, 1.05)	0.05		0.0079 (0.001, 0.01)	0.02
Baseline score		-	-		-0.3 (-0.37, -0.24)	<.0001
***C9orf72**YEO**		1.03 (0.98, 1.07)	0.23		-0.01 (-0.03, 0.007)	0.23
***GRN**YEO**		1 (0.96, 1.05)	0.96		0.0001 (-0.01, 0.01)	0.99
***MAPT**YEO**		0.94 (0.89, 1)	0.05		0.002 (-0.02, 0.02)	0.86
Motivation	587			330		
C9orf72	104	1.91 (0.72, 5.06)	0.19	49	0.093 (-0.19, 0.38)	0.52
GRN	138	0.93 (0.31, 2.75)	0.9	84	0.02 (-0.19, 0.22)	0.88
MAPT	52	3.68 (1, 13.52)	0.05[&]	31	0.0004 (-0.3, 0.3)	1
YEO		1.05 (1.02, 1.08)	0.003		0.002 (-0.0047, 0.008)	0.62
Baseline score		-	-		-0.26 (-0.33, -0.19)	<.0001
***C9orf72**YEO**		0.98 (0.93, 1.04)	0.51		0.005 (-0.0109, 0.02)	0.54
***GRN**YEO**		0.97 (0.92, 1.02)	0.26		0.006 (-0.0057, 0.02)	0.31
***MAPT**YEO**		0.97 (0.9, 1.04)	0.41		-0.006 (-0.0247, 0.01)	0.49

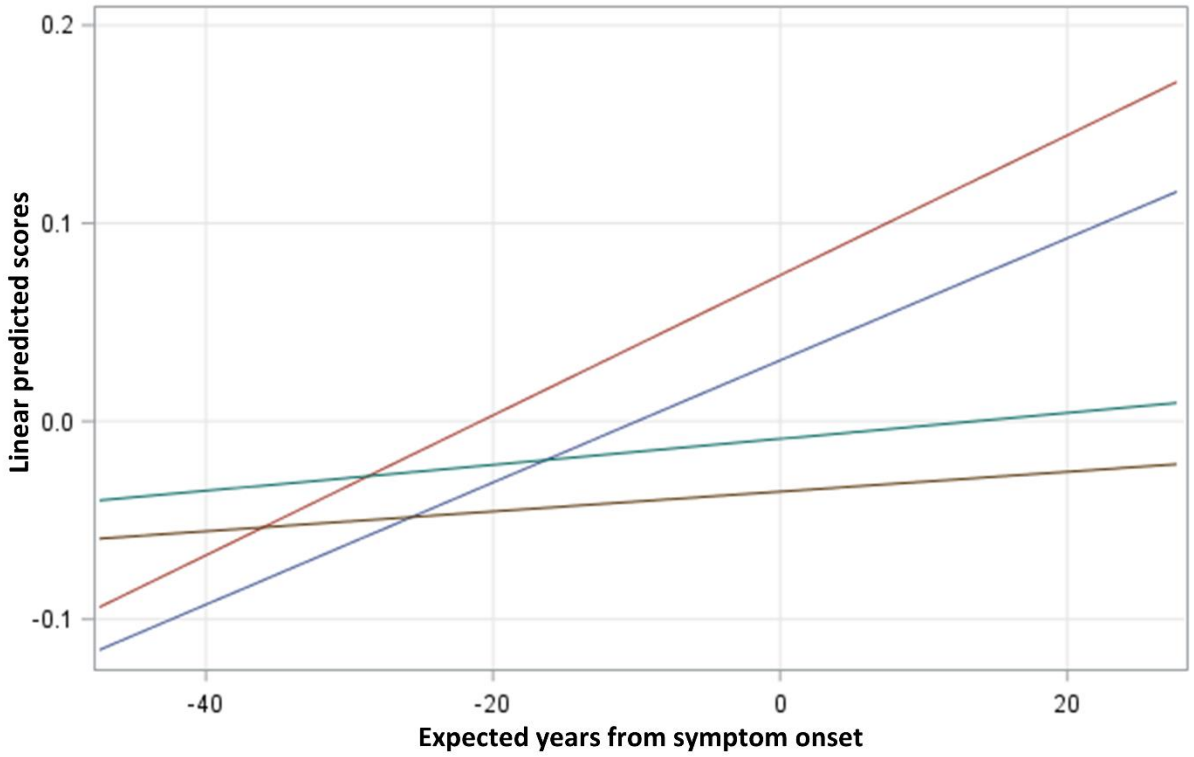
- Statistics are from the Solution for Fixed Effects Table
- #Baseline data was modeled with a negative binomial distribution with a log link function. Estimates and confidence intervals of fixed effects are exponentiated (base e) and indicate the incident rates. Estimates below 1 indicate an inverse relationship between the variable and outcome
- &Overall effect of genetic group was not statistically significant at $p<0.05$ (based on Type III Tests of Fixed Effects)
- The model could not be run on some subscales after outliers were removed due to low symptom endorsement. At baseline, for the self-care sub-scale, 3 participants (3 preclinical) had scores above zero after outliers were removed. At baseline, for the beliefs sub-scale, 4 participants (1 preclinical, 2 non-carrier) had scores above zero after outliers were removed. For the change score, for the self-care scale, 1 non-carrier endorsed a change in symptom.
- For the main effect of genetic group and Gene*EYO interaction= reference group are the non-carriers
- YEO= Years from estimated onset; CI=confidence interval

Figure 2.3. CBI-R baseline scores by years from expected onset in preclinical mutation carriers vs. non-carriers



CBI-R scores at baseline for (a) abnormal behaviours (b) mood and (c) sleep (d) stereotypic & motor (e) motivation sub-scales. Y-axis represents the scores as modeled through the generalized mixed models, and X-axis represents the expected years to onset. Blue =preclinical C9orf72 mutation carriers, red =preclinical GRN mutation carriers, green=preclinical MAPT carriers, and brown =non-carriers.

Figure 2.4. Everyday skills change score by years from expected onset in preclinical mutation carriers vs. non-carriers



CBI-R change score for everyday skills sub-scale. Y-axis represents the linear predicted scores as modeled by linear mixed models and X-axis represents the expected years to onset. Blue =preclinical *C9orf72* mutation carriers, red =preclinical *GRN* mutation carriers, green=preclinical *MAPT* carriers, and brown=non-carriers.

2.4.1 Symptomatic Period

While apathy and disinhibition were the most frequent initial symptoms across the mutation groups, some gene specific patterns emerged. The relative proportion of MAPT carriers (46%) endorsing disinhibition as the initial complaint relative to C9orf72 carriers (15%) and GRN carriers (8%) is similar to group differences previously reported where 93% of MAPT carriers exhibited signs of disinhibition over the course of their disease relative to 63% of C9orf72 and 56% of GRN carriers [9]. GRN carriers endorsed impaired articulation and

decreased fluency most often, which corresponds with the language-based clinical presentation found in some patients in this mutation group [9,13]. *C9orf72* expansion carriers reported motor symptoms most often which is consistent with reports of Amyotrophic Lateral Sclerosis found only in *C9orf72* carriers and absent in *GRN* and *MAPT* [9]. Although the symptoms discussed above are characteristic of the specific gene affected, it is critical to recognize that these symptoms are not endorsed by all the participants in each genetic group. Utilizing the top three most frequently endorsed symptom to create a composite index for each genetic group differentiated symptomatic genetic carriers from non-carriers. Future research assessing the severity of these frequently endorsed initial symptoms may aid in the differentiation between the genetic groups, and thus may be considered as an outcome measure or clinical endpoint in future clinical trials for early stage FTD.

2.4.2 Preclinical Period

Overall, and counter to our predictions, the rates of initial symptoms as endorsed by affected patients (apathy, disinhibition, memory impairments, decreased fluency and impaired articulation), were similar between preclinical mutation carriers and non-carriers. As well, the composite indices did not differentiate the groups, further supporting and extending recent findings indicating that some behavioural and cognitive changes in genetic FTD are only detectable in close proximity to conversion to the clinically affected state. Our cohort included biologically related non-mutation carriers which enabled us to control for potential environmental influences that may impact symptom endorsement (e.g. worry about inheriting an FTD-causing mutation, stress from a family member with FTD). Although biomarkers in blood and cerebrospinal fluid, grey matter atrophy, white matter hyperintensities and hypometabolism

have been detected prior to cognitive impairments during the preclinical period [1], the present findings indicate that the behavioural and cognitive symptoms endorsed as initial symptoms by patients may not emerge until just a few years prior to clear disease onset. In a recent longitudinal study of 46 preclinical mutation carriers, 8 of which "converted" to symptomatic during follow-up, cognitive decline during the preclinical period was evident but were largely driven by the converters. Additionally, differences in cognitive decline between converters and preclinical mutation carriers who did not convert was detectable starting only 2 years prior to expected onset. This may suggest that cognitive performance may remain relatively stable during the preclinical period and cognitive decline may begin near or at disease onset [14]. This finding is also consistent with a recent study that used a classification model on longitudinal MRI data (anatomical, diffusion tensor imaging and resting-state) and reported that mutation carriers who converted during follow-up had a stronger classification score increase over time relative to non-converting mutation carriers [15]. Overall, these results indicate that for some domains preclinical FTD mutation carriers may remain similar to controls until they are close to clinical disease onset.

For the caregiver report, relative to non-carriers, preclinical *MAPT* carriers endorsed poorer mood and sleep symptoms, and *C9orf72* carriers exhibited marginally greater abnormal behaviours. Moreover, *GRN* preclinical carriers endorsed fewer mood symptoms relative to non-carriers. Given the natural co-occurrence of sleep and mood alterations, it is not surprising that *MAPT* carriers experienced symptoms in both domains. In line with our current findings, depressive disorder not otherwise specified has been found to be more prevalent amongst *MAPT* preclinical carriers relative to mutation non-carriers and the general population [16]. As well, over a 4-year follow-up, it was reported that *MAPT* preclinical carriers (n=15) developed more

depressive symptoms than GRN carriers (n=31) and healthy controls (n=39) [14]. In contrast to the current study, other reports have documented inconsistent findings on the prevalence of depressive and other neuropsychiatric symptoms during the preclinical period. For example, a greater lifetime prevalence of major depressive disorder, generalized anxiety disorder and panic disorder has previously been observed in non-carriers (n=46), but not in *MAPT* mutation carriers (n=12) [16]. Furthermore, other studies have found that neuropsychiatric features may not emerge until disease onset. For example, in a Dutch cohort of approximately 80 *MAPT* and GRN mutation and non-carriers, mutation carriers who "converted" from preclinical to symptomatic status (3 *GRN* and 5 *MAPT*) displayed greater depressive and general neuropsychiatric features relative to preclinical mutation carriers and mutation non-carriers at the time of clinical disease onset [17]. In our cohort of preclinical mutation carriers, as mood symptoms did not emerge as participants approached their expected time of disease onset, the endorsement of symptoms by mutation carriers' may reflect a developmental predisposition.

When symptom endorsement was examined longitudinally, preclinical *GRN* carriers endorsed worse Everyday Skills over time compared to mutation non-carriers. Relative to healthy controls and normative data, asymptomatic *GRN* carriers demonstrate poorer performance on a variety of cognitive domains including attention/processing speed [18], visuospatial and working memory [19], verbal fluency, emotion recognition [20], attention, mental flexibility and language [21]. With this, it is likely that the decline in Everyday skills in preclinical GRN carriers reflects subtle changes in a variety of cognitive domains. Therefore, as differences are evident between *GRN* preclinical mutation carriers and non-carriers, everyday skills as measured through the CBI-R may potentially be used as an end point for clinical trials in *GRN* preclinical individuals.

2.4.3 Limitations

Potential clustering effects of family membership and testing site could not be accounted for in the clinician-rating scale, due to low symptom endorsement. As well, participant's knowledge of their genetic status was not obtained and thus this potential effect could not be accounted for. Future clinical trial modeling may need to consider the participants' knowledge of their genetic status when considering rates of symptom reporting [22]. Furthermore, although the different scales used in the current study allow for the assessment of symptom endorsement by multiple informants, we could not account for potential differences in reporting style based on the sex of the informant or the relationship of the informant to the at-risk family member. An additional potential limitation is the reliance on retrospective caregiver reports to acquire reports of the initial symptom in symptomatic mutation carriers, though the diagnosis of FTD is reliant on caregiver's reports [23].

2.4.4 Conclusions

In conclusion, we report the frequencies of the most common initial symptoms for the main genetic forms of FTD and suggest that given the heterogeneity between gene groups, family members, and even specific mutations, composite measures of these symptoms may serve as clinical tools for detection of early conversion to symptomatic FTD. Of interest, we did not find differences between preclinical mutation carriers and non-carriers for the most common initial symptoms in affected patients. Future studies examining initial symptoms with additional longitudinal data points will aid in the understanding of the progression of these symptoms from

the preclinical, to affected diseases stages and further pinpoint the onset of initial symptoms heralding conversion to symptomatic FTD.

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Chapter 3: Ventricular Volume Expansion in Preclinical Genetic Frontotemporal Dementia [2]

3.1 Introduction

Frontotemporal Dementia (FTD) is a heritable neurodegenerative disorder characterized clinically by behavioral and/or language deficits and atrophy within the frontal and temporal lobes. Approximately 30% of patients with FTD present with an autosomal dominant family history with mutations in *MAPT*, *PGRN* and *C9Forf72* each presenting in 5-25% of familial FTD [1]. Advances have been made in developing disease-modifying treatments that target the underlying pathology of FTD [2]. As the initiation of FTD-treatment is anticipated to be necessary during the preclinical or prodromal stages of the disease, biomarkers sensitive to these disease periods are needed. Brain volumetric measurements may be a promising candidate measure of disease onset and progression, as atrophy in regions of the frontal and temporal lobes may appear as early as 5 to 10 years before anticipated clinical disease onset in genetic FTD [3].

Changes in ventricular volume represent a particularly attractive candidate index of neuronal survival in FTD. Ventricular expansion is seen across the heterogenous clinical, molecular and genetic subtypes of FTD at the symptomatic stage [4,5]. Additional advantages include reduced image distortion from gradient non-linearities due to the proximity of the ventricle to the magnet's isocenter, and high contrast in intensity between ventricles and tissue which facilitates automated segmentation techniques and implementation in large clinical trials [6,7].

Ventricular expansion during the preclinical stages of genetic FTD has not yet been characterized. The objective of the present study was to examine ventricular volume expansion

[2] A version of this chapter has been published: Tavares TP, Mitchell DG, Coleman K, Shoesmith C, Bartha R, Cash DM, Moore KM, van Swieten J, Borroni B, Galimberti D, Tartaglia MC. Ventricular volume expansion in presymptomatic genetic frontotemporal dementia. Neurology. 2019; 93(18):e1699-706.

cross-sectionally and over a 1-year interval in carriers of an FTD-causing genetic mutation and biologically related non-carriers, to determine its utility as a measure of early or preclinical disease in genetic FTD.

3.2 Methods

3.2.1 Participants

The data used in this study was obtained from the multi-center international Genetic Frontotemporal Dementia Initiative (GENFI). Participants were recruited from 12 research sites across Canada, Sweden, Italy, UK and Netherlands and were either a known symptomatic carrier of a pathogenic FTD causing mutation in *MAPT*, *PGRN* or *C9orf72*, or a first-degree relative of a known symptomatic mutation carrier. As described previously [3], all participants completed clinical interviews and standardized neuropsychological testing at baseline and at 1-year follow up. At-risk first-degree relatives underwent genetic testing to determine mutation carrier status. Therefore, the sample was composed of symptomatic mutation carriers, and biologically related preclinical mutation carriers and non-carriers.

3.2.2 Imaging and Ventricular Volume Processing

Volumetric T1-weighted scans were acquired from either a 3T Philips, Siemens Trio, Siemens Skyra or GE; 1.5 Siemens or GE were utilized if a 3T was not available. Scanning protocols were designed to accommodate the different scanners and field strengths [4]. Longitudinal scans collected approximately one year after baseline, using the same scanner and protocol as the baseline scan, were included in the analysis.

The default longitudinal stream in FreeSurfer, version 5.1 (http://surfer.nmr.mgh.harvard.edu/) was used for ventricular volume processing [8]. DICOM images were converted into NIfTI format using the *mri_convert* command available in FreeSurfer. An unbiased within-subject template was created using inverse consistent registration [9]. In addition, utilizing information from the within-subject template, processing steps including skull stripping, spatial transformation to MNI space, atlas registration, spherical surface maps, and parcellations were initialized to increase reliability and statistical power [8]. Ventricular segmentations of the lateral, inferior, third and fourth ventricles were visually checked and manually edited by TPT, while blinded to familial mutation group membership, mutation status (carrier vs. non-carrier) and study period (baseline or follow-up). Volumes of the left and right lateral and inferior ventricles, third and fourth ventricles, and total intracranial volume were extracted from aseg.txt longitudinal output files.

3.2.3 Statistical Analyses

Total ventricular volume was calculated as the sum of the left and right lateral (including inferior), third and fourth ventricles and was expressed as a percentage of the individual's total intracranial volume. Main analyses compared ventricular volume in preclinical carriers vs. non-carriers, collapsed across the mutation types to maximize sample size. Exploratory analysis included genetic mutation type (*C9orf72*, *PGRN*, *MAPT*) to compare ventricular changes across the three genes.

Linear mixed models were used to examine differences in baseline ventricular volumes and change over 1-year. Preclinical carriers were compared to non-carriers to examine whether differences were detectable between asymptomatic carriers vs. non-carriers. Predictor variables in these analyses included random effects (family membership (variance components covariance structure) and each participant nested within the family (unstructured covariance structure), and a

random intercept for each) and fixed effects [visit (baseline, follow-up), genetic status (GS; carrier, non-carriers), years from expected disease onset, and an interaction term for genetic status and years from expected disease onset]. Years from expected age of disease onset were calculated by subtracting the mean age of disease onset within the family from the participant's current age at the time of baseline scan and follow up scan [3]. As ventricular volume was predicted to change in a non-linear fashion over time as individuals approached the time of expected disease onset, a quadratic term for time from expected disease onset and its interaction with genetic mutation status were included. Thus, using ventricular volume at time 1 and 2 within individuals, the model could evaluate whether a linear or quadratic change in ventricular volume was present across individuals as a function of years to expected disease onset. To create parsimonious models, non-significant interaction terms were removed. The visit by genetic status interaction was examined in a separate model without the time to expected disease onset by genetic status interaction due to multicollinearity. Residual and influence analyses were conducted to examine model quality and to identify potential outliers. Studentized and conditional residuals were examined, along with several influence diagnostic measures including Cook's D, COVRATIO, Restricted Likelihood Distance, the PRESS statistic, and MDFFIT. Given assumptions made in using the expected years to disease onset based on the average age of disease onset in the family, we also conducted a confirmatory analysis using the final model, substituting years to expected disease onset with the participants' age.

To examine differences in ventricular volume across the preclinical, prodromal and affected stages of the disease, similar models were included to compare all mutation carriers (both symptomatic, preclinical mutation carriers and progressors) relative to non-carriers. This model allows ventricular volume to be examined across the continuum of the disease and allowed an

opportunity to examine whether and how the model might change if symptomatic patients were included with preclinical carriers, as has been done in some prior studies (see Appendix B, Result Section 1.0).

Significant interactions between years from expected disease onset and genetic status were followed-up with *t*-tests to assess potential differences between the genetic carriers and non-carriers in the years prior to and after expected disease onset, across the baseline and follow-up periods. Significant results were also followed up with analysis of regional ventricular volumes (i.e. left and right). Given reported grey matter asymmetries in *PGRN* mutation carriers [10-12], we also computed and examined a laterality index, defined as the absolute difference between the left and right ventricular volumes divided by the total ventricular volume [3]. All statistical analyses were conducted using SAS® (Version 9.4).

3.3. Results

3.3.1 Participants

A total of 106 participants met the inclusion criteria. After processing in FreeSurfer, 4 participants were removed prior to statistical analysis: 1 due to scaling errors, 1 with extensive segmentation errors, and 2 found to be extreme outliers (1 carrier and 1 non-carrier from *PGRN* families; mean volumes >3 SD), leaving 102 participants from 43 family cohorts, entered into the statistical model (Table 3.1).

Table 3.1: Demographic characteristics of study participants (N=102)

	Preclinical Carrier (n=46)	Non-Carriers (n=56)	Preclinical carriers vs. Non-carriers
Genotype			p=0.74
C9orf72	13	13	
PGRN	29	36	
MAPT	4	7	
Sex			p=0.52
Female	25	34	
Male	21	22	
Years from expected disease onset at baseline Mean (SD)	-13.34(12.65)	-9.14 (15.60)	p=0.14
Age (SD)	44.66 (11.14)	50.56 (15.64)	p=0.03*
Years of education (SD)	14.20 (3.47)	14.34 (3.51)	p=0.84

Group differences were assessed using chi-square tests and t-tests. *significant at $p<0.05$

3.3.2 Preclinical Carriers vs. Non-Carriers

The final model included the genetic status by time to disease onset (linear) interaction, which illustrates differences between the genetic groups at differing points of time to disease onset. A diagnostic analysis identified two high-influential participants (non-carriers) who were subsequently removed (see Table B.8), resulting in a main effect of genetic status and a genetic status by time from disease onset interaction. Additionally, visual inspection of the scatterplot indicated an extreme case (non-carrier). Table 3.2 shows the model estimates, *p*-values and confidence intervals for the main effects [visit, genetic status, time from disease onset (linear and quadratic term)], and the interaction between time from disease onset and genetic status. Unadjusted post-hoc *t*-tests demonstrated differences in total ventricular volume between preclinical carriers and non-carriers beginning two years prior to expected disease onset, or

beginning at 4 years prior to expected disease onset when one extreme case (non-carrier) was removed from the model (Figure 3.1). Table 3.3 shows the model estimates for the total ventricular volume and total left and right volumes at specific years prior to expected disease onset (-25 years to 10 years). To create parsimonious models, we excluded sites (n=11) as a random effect and only accounted for family membership. In a confirmatory analysis, we included site as a random effect in the final model to examine the potential influence of data collection from multiple sites. Confirmatory analysis supported that site was not significant (p=0.32). In the supportive analysis, with age used instead of expected years to disease onset, a significant genetic group by age interaction was found. Post-hoc tests demonstrated that in comparison to non-carriers, pre-symptomatic carriers showed greater ventricular volume beginning at age 49.

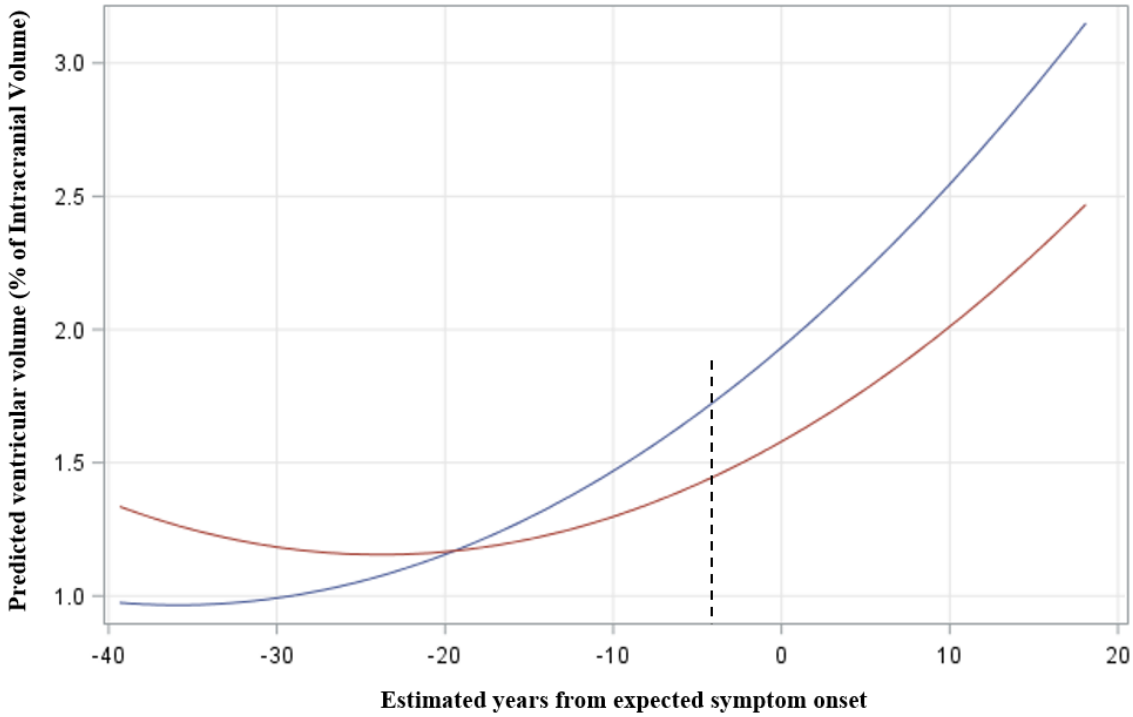
Table 3.2: Total ventricular volume estimates for preclinical (n=46) and non-carriers (n=53), with no influential cases (n=2) or extreme case (n=1)

Fixed Effects	**Estimate**	**SE**	***p*-value**	**CI (95%)**
Intercept	0.38	0.34	0.27	-0.31, 1.07
Visit (Baseline and 1-year follow-up; ref=follow-up)	0.0004	0.01	0.97	-0.02, 0.02
Genetic Status (ref=non-carrier)	0.35	0.15	0.02*	0.05, 0.66
Time from disease onset	-0.02	0.01	0.07	-0.05, 0.002
Time from disease onset^2	0.0007	0.0002	0.0001*	0.0004, 0.001
Time from symptom onset*Genetic Status	0.02	0.007	0.02*	0.003, 0.03

Random Effects	**Estimate**	**SE**	***p*-value**
Family Membership	0.02	0.04	0.33
Participant	0.37	0.07	<.0001*
Residual	0.004	0.0005	<.0001*

SE=standard error; Genetic Status (preclinical carrier vs. non-carrier); CI=Confidence interval**;** *****significant at $p<0.05$; Time from disease onset^2= Quadratic term for time from disease onset variable; ventricular volume is presented as a percentage of the participant's total intracranial volume; The interaction between time from disease onset and genetic status illustrates differences between the genetic groups (preclinical carriers vs. non-carriers) at differing points of time to disease onset.

Figure 3.1: Total ventricular volume by estimated years from expected disease onset in pre-symptomatic carriers (blue, n=46) and non-carriers (red, n=53).



Ventricular volume is expressed as a percentage of intracranial volume. To prevent disclosure of genetic status, individual data points are not plotted. Differences are noted beginning at 4 years prior to disease onset as indicated by the dashed line (p=0.04).

Table 3.3. Total ventricle volume estimates from post-hoc test between preclinical mutation carriers (n=46) and non-carriers (n=53) by time to expected disease onset with no influential cases (n=2) or extreme case (n=1)

	-25 years	-20 years	-15 years	-10 years	-5 years	0 years	5 years	10 years
Total Ventricle								
Estimate	-0.10	-0.010	0.08	0.17	0.26	0.35	0.44	0.53
SE	0.16	0.14	0.13	0.13	0.14	0.15	0.18	0.20
p-value	0.54	0.94	0.53	0.18	0.05	0.02*	0.01*	0.01*
Total Left Ventricle								
Estimate	-0.07	-0.02	0.03	0.08	0.13	0.18	0.23	0.28
SE	0.08	0.07	0.06	0.06	0.06	0.07	0.08	0.10
p-value	0.39	0.80	0.60	0.18	0.05*	0.02*	0.01*	0.01*
Total Right Ventricle								
Estimate	-0.05	-0.01	0.03	0.07	0.11	0.15	0.19	0.23
SE	0.09	0.07	0.07	0.07	0.07	0.08	0.09	0.11
p-value	0.59	0.94	0.62	0.28	0.12	0.06	0.04*	0.03*

*****significant at $p<0.05$; ventricular volume is presented as a percentage of the participant's total intracranial volume.

3.3.3 Manually Edited Versus Fully Automated Ventricular Volumes

Manual edits to the ventricular segmentations performed in FreeSurfer were made on all study participants for each time point (mean differences in edited vs. unedited volumes are reported in supplementary analysis). Substitution of the fully automated ventricular volumes produced by FreeSurfer into the final models resulted in similar findings, demonstrating that for preclinical carriers vs. non-carriers, significant differences were observed at 4 years prior to disease onset (Tables B.9a-b in Appendix B). See Table B.6 for annualized change of unedited ventricular volume.

3.3.4 Total Ventricular Expansion over 1 year

To assess potential differences in ventricular expansion over the 1-year interval, an additional model was evaluated that included the same family and participant random effects as above and the following fixed effects: visit, years to disease onset (linear and quadratic terms), genetic status and an interaction between visit and genetic status. Significant visit by genetic status interaction was followed-up by simple effects estimation. There was a significant time by genetic status interaction ($p=0.03$); however, follow-up tests did not reach significance (all p's> 0.18). Annual rates of change of total ventricular volume are presented in Table.7, as a function of genetic status and years to expected disease onset.

3.3.5 Mutation Type

Given previously reported differences in atrophy patterns across the different genotypes, we conducted an exploratory analysis to assess potential differences in total ventricular volume

and in the laterality index between the genotypes. Specifically, utilizing the final models from previous analysis (with the extreme case and two influential cases), we included genotype (*C9orf72, PGRN, MAPT*) and the interaction between genotype and genetic status as fixed effects in the model. There was no significant interaction between genotype and genetic status (p=0.10). There was no significant interaction between genetic status and genotype for the laterality index (p=0.63).

3.4 Discussion

In this multi-center cohort of individuals from families with genetic FTD, we found that ventricular volume enlargement is detectable in the preclinical period, on average four years prior to the anticipated onset of symptoms. We also provide the first estimates of annualized rates of ventricular expansion in preclinical gene carriers compared to biologically related non-carriers.

We also examined ventricular volume changes across the preclinical and prodromal to symptomatic stages of the disease, which offers a unique opportunity to explore ventricular volume changes throughout the disease continuum. This method has also been employed by other studies [3] delineating cross-sectional grey matter volumes in a genetic cohort. When all mutation carriers (symptomatic & preclinical) were included in the model, ventricular volume changes emerged 12 years prior to disease onset. This is in contrast to the model including only preclinical participants. Importantly, the preclinical model allows the examination of subtle changes that emerge a few years prior to disease onset, unbiased by the increased rate of change that may occur during the symptomatic period. Thus, the different model estimates and the earlier detection of ventricular volume changes in the combined model of symptomatic patients with the preclinical individuals likely reflects the increased the rate of change of ventricular volume as the disease progresses. We suggest that the preclinical carrier vs. non-carrier model identifying

measurable differences 4 years prior to anticipated disease onset offers the more accurate depiction of ventricular volume changes throughout the preclinical period.

Ventricular volume expansion has been well documented in patients with symptomatic sporadic and genetic forms of FTD [13-15]. The annual mean rates of expansion for symptomatic mutation carriers in this GENFI cohort rate range from 6-11% and are in line with those reported in a series of 6 symptomatic *C9orf72* carriers (mean annualized rate of ventricular expansion of~9%) [14] and in 21 *MAPT* symptomatic carriers (~9%) [16]. The expansion rate is slightly lower than that reported previously in a cohort of patients with sporadic FTD (mean 11-14%) [13] despite similarities in the mean age of symptomatic participants. Prior small series of preclinical mutation carriers have not detected significant differences in ventricular expansion rates in 7 *C9orf72* preclinical carriers (mean age 41) over a six month interval [17], or in 9 preclinical *MAPT* carriers (1 year interval) [16].

The varied and dramatic atrophy patterns observed in FTD can introduce difficulties for automated segmentation programs [18]. We focused our analysis on total ventricular volume in particular, as in the preclinical state, the laterality and exact brain regions that may display the earliest signs of atrophy are not certain, even within a genetic mutation. Such advantages of whole brain measurements, such as total ventricular volume, for tracking outcomes have been previously described [13,19,20]. Despite theoretical concerns about lesser sensitivity due to averaging across brain regions, this study supports the potential for total ventricular volume measurements to provide an unbiased approach to capture accelerated rates of atrophy in preclinical and early symptomatic stages of disease. Our comparison of time-intensive, detailed manual editing of all scans included in the study relative to the fully automated segmentation with no editing produced remarkably similar results, further supporting the feasibility of total ventricular volume as a practical measure of disease onset and progression in multi-center clinical

trials in FTD.

Despite the relatively large sample for a cohort of preclinical FTD mutation carriers, subgrouping by mutation type and years to expected disease onset resulted in significant variability in some estimates. While significant variability in rates of atrophy has been reported within mutation groups and even within families [21], in the current study, the variability may potentially be due at least in part to the sample size of subgroups. Due to subgroup sample size, the examination of differences between carriers of *C9orf72, PRGN* and *MAPT* was exploratory, and did not reach significance. Examination of subregions of ventricular expansion identified the third ventricle as one of the earliest markers in symptomatic patients (Appendix A, supplementary analysis). While we did not identify a significant interaction between genetic status and time to expected disease onset for the third ventricle in preclinical carriers, inspection of the post-hoc tests indicate early expansion of the third ventricle (~14 years prior to expected disease onset) in this cohort as well. Together these findings suggest that enlargement of the third ventricle in preclinical *C9orf72* carriers may be one of the first neuroimaging derived markers, due to early thalamic atrophy [22].

An additional potential limitation of this study is the use of the estimated age at disease onset, calculated by subtracting the mean age of disease onset within the family from the participant's age at the time of testing. While previous work has demonstrated a strong association between patient's age at disease onset and mean familial age at disease onset [3], it has been observed that age of disease onset within families is particularly variable in *GRN* mutations and somewhat variable in *C9ORF72*. Although we found similar results when current age or actual age at disease onset was substituted into the models, we cannot yet confirm how accurately the anticipated age at disease onset represents the actual age in the majority of individuals in the pre-symptomatic cohort. Data anticipated from collaborations across large FTD

cohorts including LEFFTDS, ARTFL, GENFI and DINAD examining individual age at disease onset with family age of disease onset, parent age of disease onset, and other potential mediator factors will be helpful in the future to improve such models. Furthermore, differences between the pre-symptomatic carriers and non-carriers were based on group-wise estimates and could not yet be applied on an individual basis. Data collection of additional longitudinal timepoints continues for the GENFI cohort which will inform future estimates of individual rates of change in ventricular volumes.

Overall, the present study shows ventricular volume differences during the preclinical period in genetic FTD pathophysiology and support the potential of application of ventricular volume as one index of disease onset in the prodromal stages of FTD. Future longitudinal follow up of this GENFI cohort, as well as comparison with anticipated results from other familial FTD cohorts such as LEFFTDs (https://memory.ucsf.edu/lefftds), and with complementary measures such as rates of change in total brain volume will enable further modeling according to specific genotype and confirm the rates of change.

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Chapter 4: Neural Correlates of Reversal Learning in Frontotemporal Dementia

4.1 Introduction

Frontotemporal Dementia (FTD) is a progressive neurodegenerative disorder that primarily affects the frontal and temporal lobes, resulting in profound alterations in personality and social behaviour (Neary, 1998). Disinhibition is one of the core symptoms in FTD and emerges quite early during the disease process [1]. Patients with disinhibition often lack social etiquette (e.g. making rude comments), engage in impulsive or careless actions (gambling, overspending), or new criminal behaviours (e.g. shoplifting). [1,2]. Importantly, despite negative social and legal consequences, patients with FTD continue to engage in these behaviours, implicating a possible difficulty in flexibly altering behaviour when provided negative feedback. In patients with FTD, disinhibition as reported by caregivers has been correlated with degree of atrophy or hypoperfusion in frontotemporal regions, most commonly in the orbitofrontal cortex, inferior frontal gyrus, anterior cingulate gyrus, and insula [3,4]. Despite knowledge of these anatomic associations with symptoms of disinhibition, effective treatments for such symptoms in patients with FTD are lacking. Here we propose to demonstrate the neural mechanisms underlying these symptoms by examining the real-time neural correlates of reversal learning, a classic cognitive paradigm that indexes flexible responding to negative feedback and for which the mediating neural regions are well established.

Reversal learning is a classic measure of adaptive behaviour flexibility, specifically assessing the ability to alter behaviour when reinforcement contingencies change [5]. Through trial and error, participants learn stimulus-reward contingencies (*acquisition phase*), selecting stimuli associated with reward and avoiding stimuli associated with punishment. During the *reversal phase*, the reinforcement contingencies change, such that the stimuli that were previously associated with punishment are now associated with a reward, and those initially

associated with punishment are now rewarded. Functional neuroimaging studies have implicated the ventromedial prefrontal cortex/orbital frontal cortex (vmPFC/OFC), ventrolateral PFC (vlPFC) and dorsomedial PFC (dmPFC) during reversal learning. Specifically, when a reversal error is made, the vmPFC/OFC demonstrates a decrease in activity [6,7], suggesting a role in prediction error signalling which signals discrepancy between the expected and actual reward [8]. In contrast, the vlPFC, dmPFC and dlPFC demonstrate increased activity in response to reversal errors [6,9-12]. Consistent with the suggested role of the dlPFC in attention and cognitive control during instances of conflict such as during reversal errors, the dlPFC augments the representation of relevant stimulus cues and reinforcement information to guide flexible behaviour [12,13]. As well, it has been suggested that during reversal learning the vlPFC interacts with the caudate to increase the salience of alternative motor responding [6,12].

In patients with ventral frontal lesions, deficits in reversal learning are associated with increased behavioural problems including disinhibition, irritability, inflexibility and perseveration (Rolls et al, 1994). Patients with FTD exhibit reversal learning impairments [14-16], and the number of reversal errors has been correlated with atrophy within the anterior cingulate cortex (ACC; BA 24) and the medial/lateral OFC (BA 11, 47) [15]. This finding suggests that reversal learning impairments in FTD may be related to a failed suppression of a previously rewarded response mediated by vlPFC and ACC, and/or impaired processing of unexpected negative feedback by ventromedial PFC. Critically, no study has delineated the functional neural correlates of reversal learning performance in patients with FTD. Assessing the functional integrity of the key regions involved in reversal learning may provide insights into the fundamental pathophysiological mechanisms underlying behavioural inflexibility in FTD.

Furthermore, the results of the current study may have implications in the selection of therapeutic targets and future outcome markers for symptomatic treatments.

4.2 Methods

4.2.1 Participants

Twenty-seven patients with FTD and 24 controls were enrolled in the study. Five patients were unable to complete the scan and withdrew, 2 controls withdrew due to claustrophobia, and 1 control participant's data was unusable due to computer problems resulting in 22 patients and 21 controls with fMRI scans available for analysis. All patients met the diagnostic criteria for probable or definite bvFTD or svPPA with significant behavioural features [1,17]; diagnoses were made by a behavioural neurologist (ECF). Patients were recruited through the Cognitive Neurology and Alzheimer's Research Centre at Parkwood Hospital in London, Ontario, Canada, and control participants were recruited through advertisements to caregivers at local FTD support groups and from the clinic's volunteer pool. All participants and caregivers of patients provided written informed consent. This study was approved by the Health Science Research Ethics Board at Western University, London, Ontario, Canada.

4.2.2 Measures

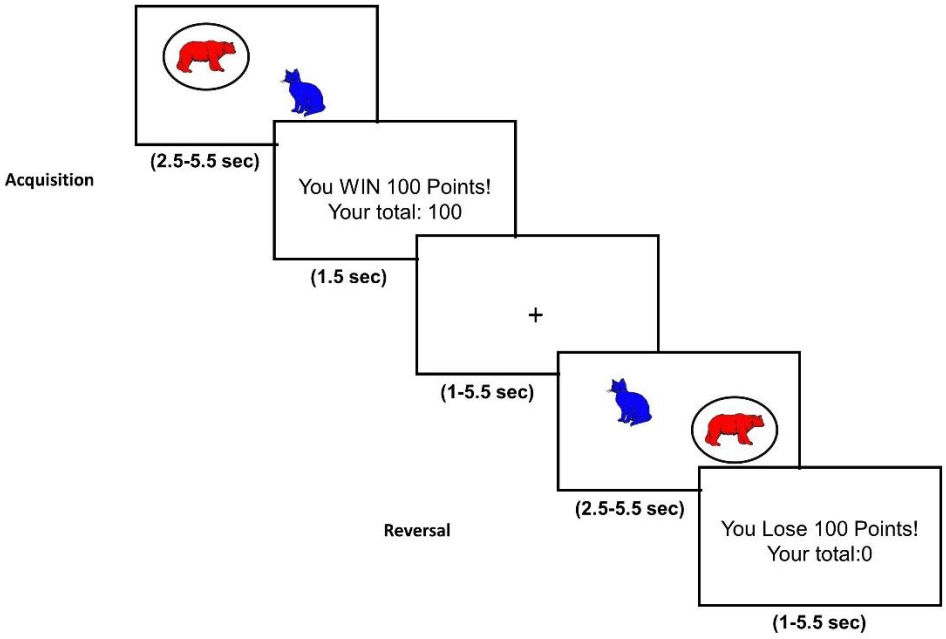
To assess cognitive functioning, patients and controls completed the Addenbrooke's Cognitive Examination-Revised (ACE-R) [18], Montreal Cognitive Assessment [19], Trail Making Test, Clock drawing [20], Prose recall, and Phonemic and Semantic Fluency. Additionally, caregivers of patients completed the Frontal Behavioural Inventory with a trained research assistant (FBI) [21]. The FBI is a 24-item inventory used to assess behaviour and

personality changes and each item is rated on a 4 point rating scale where higher scores indicate more severe behavioural changes (0=none; 1=mild, occasional, 2=moderate; 3-severe, most of the time).

4.2.3 fMRI Task

Participants completed an fMRI-adapted deterministic reversal learning task (Figure 4.1) which consists of acquisition and reversal phases. A deterministic design was selected based on our prior piloting in patients with FTD where we observed significant impairment in learning the initial reinforcement associations when probabilistic feedback was given. For the current study, on each trial, participants were presented with a pair of objects (colored Snodgrass images of animals or furniture pieces [22] and were instructed to select one of the objects in each pair. Participants were informed if their response selection was correct, they would win 100 points, and conversely, if their response selection was incorrect they would lose 100 points (acquisition phase). Subsequently participants were instructed that at some point during the task, the object in the pair associated with a correct response may change, and they should adjust their response accordingly. All participants completed practice trials outside of the scanner to ensure comprehension of task instructions.

Figure 4.1: Schematic of fMRI reversal learning task.



Schematic of the reversal learning task. During the acquisition phase, patients learn the stimulus-response contingencies. During the reversal phase, the stimulus-response contingencies would change

Participants completed four runs of the task; each run consisted of a reversal block (10 or 15 trials of acquisition phase, followed by 10 or 15 trials of reversal phase), and a non-reversal block (consisting of 20 trials of acquisition phase only). Non-reversing blocks were included to prevent participants from anticipating a contingency change across the runs. The order of the blocks was randomly presented in each run, and each run and block consisted of new stimuli. On each trial, the participants were presented with the response screen depicting the pair of objects for varying durations (2500ms, 4000ms, or 5500ms), the feedback screen (1500ms) and the fixation cross presented in the center of the screen (for 1000ms, 2500ms, or 5500ms). One of each object in a pair was presented randomly on the left and right side of the screen at 8 possible

locations. Participants responded using their index finger on their left and right hand to select the button that corresponded to the location of the correct object (left or right).

4.2.4 Imaging Acquisition

All participants completed the imaging task using a 3T Siemens Magnetom Prisma scanner with a 32-channel head coil at Robarts Research Institute, Western University. Functional images were acquired using a T2*gradient echo-planar imaging sequence [repetition time (TR)= 2500ms, echo time (TE)= 30ms, field of view (FoV)= 240mm, flip angle =90 degrees, 37 interleaved slices of 2 x 2x 3mm voxel resolution, 3mm slice thickness, 153 volumes per run]. In addition, a high resolution T1-weighted anatomical scan was obtained subsequent to the functional scan: [TR= 2300ms, TR= 2.98ms, FoV= 256mm, flip angle =9 degrees, 192 interleaved slices of 1mm isotropic voxels].

4.3 Statistical Analysis

4.3.1 Task Behavioural Performance

For each task phase (acquisition, reversal), the percentages of correct responses and non-responses as a function of the total number of trials per phase were analyzed. Visual inspection of histogram and Q-Q plots indicated non-normal data; thus, a Mann Whitney U Test and a Wilcoxon Signed-Rank Test was conducted to examine differences between and within the patient and control groups, respectively.

Behavioural performance was also examined within the patient group based on the different atrophy patterns (frontal vs. temporal predominant). Patient's MRI images were classified according to their predominant atrophy pattern by a behavioural neurologist (ECF),

resulting in 8 patients with frontal predominant atrophy, 10 with temporal predominant atrophy, and 1 with frontotemporal atrophy. To delineate potential differences between frontal and temporal atrophy patterns specifically, the patient with frontotemporal atrophy was not included in the analysis. Given the small sample size of the sub-groups, Kruskal-Wallis and Dunn-Bonferroni post-hoc tests were used as an exploratory analysis to compare behavioural performance across the frontal and temporal predominant atrophy groups and controls.

4.3.2 Frontal Behavioural Inventory (FBI)

We examined whether patients with different atrophy patterns (frontal vs. temporal predominant) exhibited differences in caregiver reported behaviours associated with reversal learning deficits [5]. Specifically, a reversal learning composite score was created from the following FBI items: inflexibility, perseverations/obsessions, impulsivity/poor judgement, irritability, and aggression. A Mann Whitney U Test was used to examine differences between the patient atrophy groups.

4.3.3 Imaging

4.3.3.1 fMRI

Imaging data were preprocessed and analyzed in Analysis of Functional Neuroimages (AFNI) [23]. Nonlinear registration was completed to the MNI template using AFNI's @SSwarper. Additionally, all volumes were registered to the functional volume closest to the anatomical (last volume of the final run). Three participants' anatomical images were acquired prior to the functional runs, thus the 3rd EPI volume of the first run was used for registration. Volumes were spatially smoothed using a 4mm full width at half maximum isotropic Gaussian

kernel. Additionally, time series data were normalized by dividing the signal intensity of voxels at each time point by the mean signal intensity of that voxel for all the runs and multiplying it by 100; thus, the regression coefficients represented the percent signal change from the mean activity. Within task runs, volumes were censored if the derivatives of the six generated motion parameters had a Euclidean norm greater than 2.0mm. To further account for movement, motion derivates were included as regressors in the model. For the voxel time series, TRs with at least 10% of voxels too far from the trend (calculated using AFNI's mean absolute deviations) was deemed an outlier and removed. Regressors characterized the response type (correct or incorrect) by phase type (acquisition or reversal) were created for both the choice (stimuli presentation/decision-making phase) and feedback screens and for reversing pairs and non-reversing pairs, resulting in 24 regressors. The blood-oxygen level dependent (BOLD) response was fitted to each regressor to conduct linear regression modelling. To account for voxel-wise correlated drifting, a baseline and linear drift and AFNI's default polynomial trend were modelled to the time series of each voxel. Linear regression modelling resulted in a beta-coefficient and an associated *t*-statistic for each voxel for each regressor. To correct for multiple comparisons, AFNI's updated 3dClustSim at a cluster defining threshold of p=0.05, was used on the entire brain with a threshold of p<0.001 (22 contiguous voxels).

Three patients were removed due to excessive movement; thus 19 patients and 21 controls were included in the behavioural and functional analysis. Additionally, one patient only completed three runs and thus a comparative run was removed from a control scan in order for both groups to have the same number of trials. A 2 Group (patients vs. controls) x 2 Phase (acquisition x reversal) x 2 Response accuracy (correct vs. incorrect) repeated measures ANOVA was conducted in AFNI using 3dMVM, and the significance threshold was set to p<0.001.

Significant interactions were followed by uncorrected paired and independent t-tests in SPSS at $p<0.05$ to examine differences in percent BOLD signal change. As an exploratory analysis, voxel-wise grey matter tissue probabilities were entered as a covariate in the fMRI analysis to account for grey matter volume differences that may influence the fMRI signal (see Method C.1, Table C.1, Table C.2, Figure C.1).

4.3.3.3 Region of Interest Analysis

An exploratory region of interest (ROI) analysis was conducted to examine whether patients with different atrophy patterns (frontal vs. temporal predominant) demonstrated different BOLD signal during the choice and feedback epochs in the regions known to be involved in response reversal. An ROI approach was used instead of an ANOVA with group included as a between-subject factor, due to the small sample in each atrophy group.

Anatomical ROIs were created using the *CA_ML_18_MNIA* atlas in AFNI. For the choice epoch, ROIs included the left vlPFC (orbitalis), and left dorsolateral PFC (triangularis/opercularis) that encompassed the clusters found below. A 3 group (frontal, temporal, control) x 2 response accuracy (correct, incorrect) x 2 phase (acquisition, reversal) and a 3 group (frontal, temporal, control) x 2 response accuracy (correct, incorrect) was used to delineate group effects in the left vlPFC and left dlPFC, respectively. One-way ANOVAs were completed to delineate group differences.

For the feedback epoch, the following ROIs were used to partition the large clusters found (see results below), into specific regions known to be uniquely involved in reversal learning [6,9,10]: left and right ventrolateral PFC, dorsolateral PFC, ventromedial PFC (superior orbital gyrus, rectal gyrus and mid orbital gyrus), and dorsomedial PFC (anterior and middle

cingulate cortex). A 3 group (frontal, temporal, controls) x 2 response accuracy (correct, incorrect) repeated measures ANOVA was completed to identify group differences for the ROIs. Uncorrected one-way ANOVAs and paired t-tests for each group were completed to delineate differences in BOLD activity between correct and incorrect response feedback.

4.4 Results

4.4.1 Demographic

Nineteen patients and 21 controls were included in the behavioural and functional imaging analysis. At the time of testing, patients were on average 63 years of age and controls were 64 years of age. Patients were diagnosed at a mean age of 62. Ten patients were diagnosed with behavioural-variant FTD (bvFTD), seven patients were diagnosed with semantic dementia (SD), one patient presented with progressive nonfluent aphasia (PNFA), and one patient presented with bvFTD/SD (Table 1 for demographic and neuropsychological performance). There were no significant differences for age at testing, sex, handedness, or years of education between the patients with FTD and controls.

Table 4.1: Demographic and Neuropsychological Characteristics

Demographic	**Patients**	**Controls**	**Contrasts**
	19	21	
Age at testing, years (SD)	63.3 (5.4)	64.1 (13.9)	t(26.5)= -0.23. p=0.82
Sex (Male, Female)	10, 9	10, 11	X^2=0.1, p=0.8
Handedness (left, right)	0, 19	2, 18*	Fisher's = 2.5, p=0.5
Education (years)	13.8 (1.8)	14.4 (2.8)	t(38)= -0.77, p=0.45
Age at diagnosis (SD)	61.6 (5.6)	-	-
Disease duration, Yrs (SD)	1.8 (1.9)	-	-
Atrophy Pattern		-	-
Frontal	8	-	-
Temporal	10	-	-
Frontotemporal	1	-	-
Diagnosis		-	-
bvFTD	10	-	-
SD	7	-	-
PNFA	1	-	-
SD & bvFTD	1	-	-

Neuropsychology	**Patients**		**Controls**		**Contrasts**
	n	**Mean (SD)**	**n**	**Mean (SD)**	
MoCA	18	19.0 (5.0)	18	26.9 (2.0)	t(22)= -6.3, *p*<0.001
ACER (total)	19	64.2 (17.8)	21	88.7 (21.1)	t(38) = -3.9. *p*<0.001
Prose immediate Recall	16	4.1 (3.8)	18	10.72 (3.1)	t(32)= -5.6, *p*<0.001
Prose delayed recall	18	3.2 (3.7)	18	10.3 (2.6)	t(32) = -6.5, *p*<0.001
Semantic fluency	19	10 (5.9)	18	22.0 (4.2)	t(35) = -7.1, *p*<0.001
Phonemic fluency	17	21.6 (14.4)	19	40.5 (12.4)	t(34) = -4.2, *p*<0.001
Trail A	18	50.7 (15.0)	18	27.6 (7.5)	t(25)= 5.9, *p*<0.001
Trail B	14	121.3 (58.5)	18	63.3 (16.4)	t(14.6)= 3.6, *p*=0.003
Clock draw	18	8.2 (2.5)	18	9.6 (0.8)	t(20.3) = -2.4, *p*=0.03
Clock (copy)	16	9.3 (1.1)	17	9.7 (0.5)	t(19.7)= -1.3, *p*=0.2
FBI Composite Score	19	6.4 (3.5)	-	-	-

*Handedness was absent for one control participant
bvFTD=behavioural variant FTD; SD=semantic dementia; PNFA=progressive non-fluent aphasia; MoCA=Montreal Cognitive Assessment; ACER= Addenbrooke's Cognitive Examination; FBI=Frontal Behavioural Inventory

4.4.2 Task Behavioural Performance

4.4.2.1 Patients vs. Controls

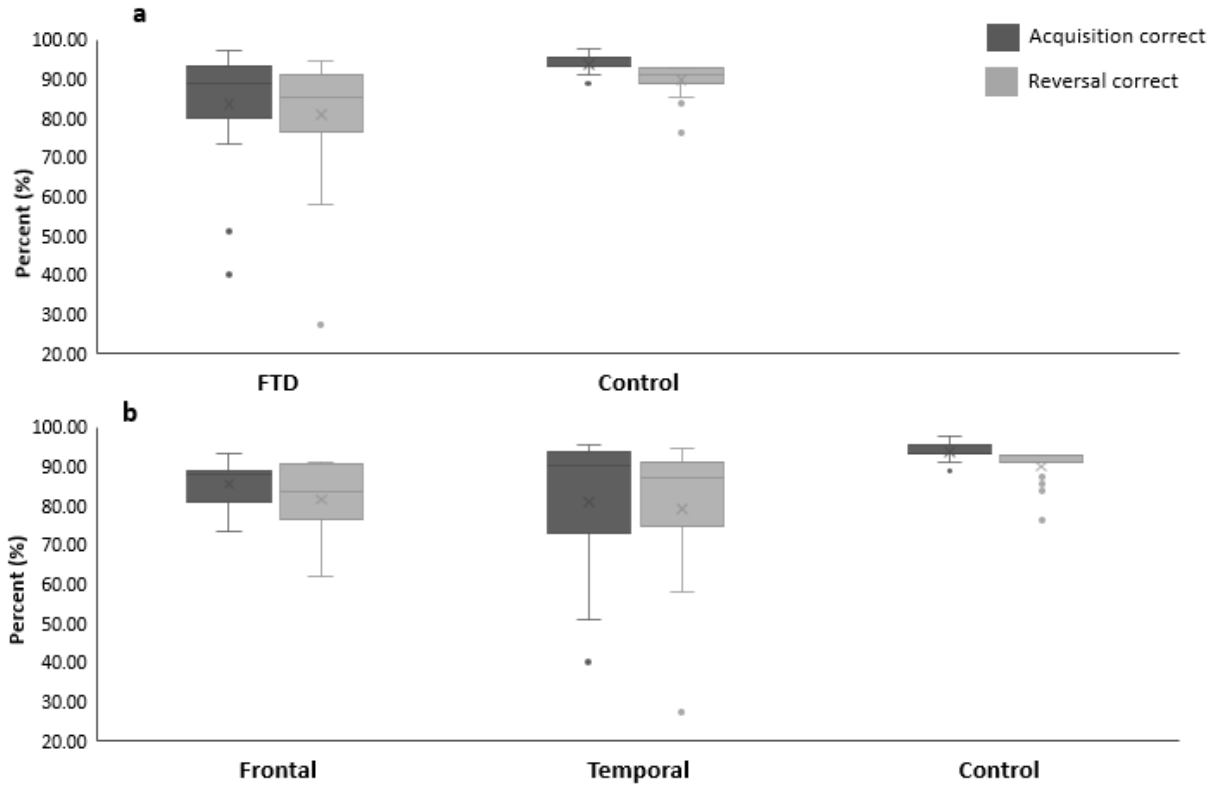
The control group made more correct responses relative to patients during the acquisition phase (U=81.0, p=0.001) and reversal phase (U=95.5, p=0.004). Of note, two patients had 51% or fewer correct responses during acquisition and reversal phase; when removed from the analysis the group differences remained (Acquisition: U=81, p=0.003; Reversal: U=95.5, p=0.012).

As expected, controls made more correct responses during the acquisition relative to the reversal trials (Z= -3.2, p=0.002). Patients showed a trend to make more correct responses during the acquisition relative to the reversal stage (Z= -1.9, p=0.06Figure 4.2a; Table C.3).

4.4.2.2 Frontal predominant atrophy, temporal predominant atrophy and controls

Controls made more correct responses relative to the temporal (Z=-2.48, *p*=0.04) and frontal atrophy (Z=-3.42, *p*=0.002) group during acquisition (Mean ranks: Control = 25.5, Temporal= 15.1, Frontal=10.0). During reversal, controls made more correct responses relative to the frontal group (Z=-2.79, *p*=0.02); no differences were found between the control and temporal group (Z= -2.09, *p*=0.11; Mean ranks: Control=24.5, Temporal=15.7, Frontal=11.9; Figure 4.2b).

Figure 4.2: Percent correct responses during acquisition and reversal trials



Boxplots of percent correct and non-responses during acquisition and reversal phases for (a) patients and controls and (b) frontal, temporal atrophy groups and controls. (a) For the acquisition and reversal phase controls made more correct responses relative to patients. Patients made more non-responses relative to controls. (b) During acquisition, controls made more correct responses relative to the temporal and frontal groups.

4.4.3 FBI

Patients with temporal and frontal atrophy did not exhibit differences in the FBI-reversal learning composite score (U=34.0, p=0.63).

4.4.4 fMRI

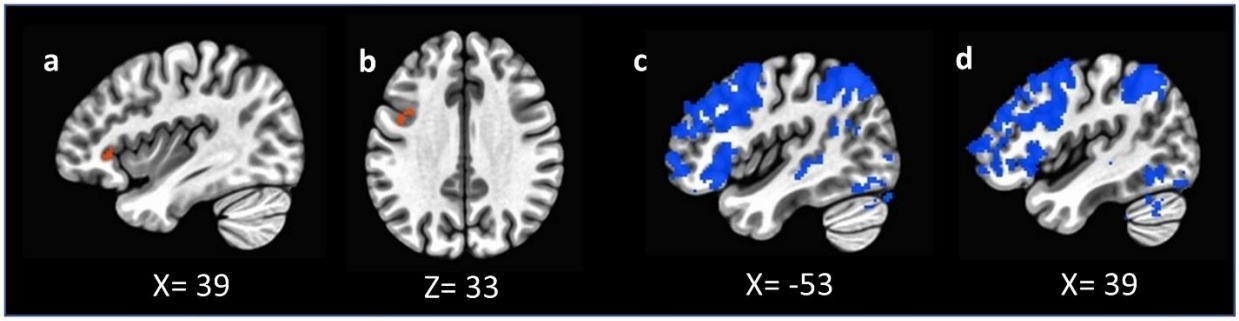
4.4.4.1 Choice Epoch

The repeated measures ANOVA of BOLD signal during the choice phase revealed a significant 3-way interaction within the left vlPFC/anterior insula (Table 4.2), where controls exhibited greater activity relative to patients during acquisition error responses (t= (38), -4.4, $p<0.0001$) and reversal correct responses (t= (38), -3.6, $p=0.001$; Figure 3a). Controls showed greater activity in vlPFC/anterior insula during acquisition for incorrect relative to correct responses (t=(20), -4.6, $p<0.001$), while in patients BOLD signal did not differ for acquisition correct vs. incorrect responses (t= (18), 1.0, $p=0.31$). During reversal, in this region the opposite pattern emerged, where controls did not exhibit a difference in BOLD activity between the response accuracy (t= (20), 1.5, $p=0.16$), while patients demonstrated greater activity for errors relative to correct responses (t= (18), -2.22, $p=0.04$). Additionally, a group x response accuracy interaction emerged where controls exhibited greater BOLD activity during incorrect responses relative to patients within the left dorsal lateral PFC (t= (38), -4.6, $p<0.001$; Table 4.2, Figure 3b). There was also a significant interaction between phase and response accuracy within the dorsal lateral and medial PFC, vlPFC, and striatum (see Table 4.2).

4.4.4.2 Feedback Epoch

A main effect of response accuracy was observed in two large clusters that encompassed the right lateral and ventromedial PFC, right insula and bilateral dmPFC (cluster 1), and left insula and left ventromedial and lateral PFC (cluster 2) where activity was greater for incorrect relative to correct responses (Figure 4.3c-d, Table 4.2).

Figure 4.3: Neural regions demonstrating significant effects during choice and feedback epochs



Group x phase x response accuracy during the choice epoch in the left vlPFC/anterior insula for controls versus patients (a), group x response accuracy interaction in the left dlPFC for controls versus patients (b), main effect of accuracy for incorrect versus correct responses for the right (c) and (d) left lateral PFC.

Table 4.2: Regions demonstrating differential BOLD Signal Responses

Choice Phase									
Fixed Effects	**Region**	**L/R**	**BA**	**X**	**Y**	**Z**	**t-value**	**# voxels**	**Direction**
Group	Middle temporal gyrus	L	22	60.4	32.1	3.8	-4.1	57	Ctrl>Pat
	Inferior frontal gyrus	L	47	32.4	-28.5	0.1	-4.0	50	Ctrl> Pat
	Inferior frontal gyrus	R	9	-53.4	-4.3	35.8	-4.6	27	Ctrl > Pat
Reinforcement	Inferior frontal gyrus	L	6/9	41.3	-0.7	33.2	-4.3	27	Corr<incorr
Phase	Left inferior frontal gyrus	L	9	43.1	-4.9	34.6	4.1	276	Acq > Rev
	Left medial frontal gyrus	L	6	5.6	-15.4	48.8	4.8	229	Acq > Rev
	Lentiform nucleus & putamen	L		17.4	-8.9	-2.4	4.0	221	Acq > Rev
	Inferior frontal gyrus	L	47/13	31.2	-25.4	-3.2	3.4	130	Acq > Rev
	Inferior frontal gyrus	R	9	-39.6	-6.8	31.4	4.4	110	Acq > Rev
	Lentiform nucleus/putmane	R		-15.0	-7.6	-1.6	3.0	95	Acq > Rev

	Inferior frontal gyrus	R	47	-30.7	-23.2	-2.6	4.2	76	Acq > Rev
	Cingulate gyrus	R	32	-9.2	-17.9	38.4	4.2	72	Acq > Rev
	Superior occipital gyrus	R	19	-31.9	77.3	22.7	3.8	69	Acq > Rev
	Inferior parietal lobe	L	40	33.1	48.5	43.7	2.5	34	Acq > Rev
	Superior parietal lobe	L	7	29.8	56.8	49.9	5.1	33	Acq > Rev
	Inferior parietal lobe	L	40	53.0	45.2	49.0	5.4	24	Acq > Rev
	Precuneus	L	39	28.6	68.4	31.4	3.8	23	Acq > Rev
Phase x reinforcement	Cingulate gyrus	L	32	3.9	-20.9	45.5	-3.1	253	Acquisition Corr<Incor Reversal Corr>Incorr
	Inferior frontal gyrus	L	9	44.0	-3.1	35.9	-3.6	208	Acquisition Corr< Incorr Reversal Corr>Incorr
	Lentiform nucleus/Putamen	L		17.3	-9.4	-2.9	-3.7	199	Acquisition Corr< Incorr Reversal Corr>Incorr
	Inferior frontal gyrus	L	47	34.3	-25.7	-5.1	-4.8	96	Acquisition Corr< Incorr Reversal Corr>Incorr
	Lentiform nucleus/putamen	R		-14.4	-8.2	-3.6	-4.5	51	Acquisition Corr< Incorr
	Middle frontal gyrus	L	46	42.9	-28.3	21.8	-4.8	51	Acquisition Corr< Incorr Reversal Corr>Incorr
	Cingulate gyrus	R	32	-7.7	-16.8	41.3	-3.8	46	Acquisition Corr< Incorr Reversal Corr>Incorr
	Inferior parietal lobule	L	40	34.1	51.7	43.1	-2.8	40	Acquisition Corr< Incorr Reversal Corr>Incorr
	Cingulate gyrus	L	32	10.4	-19.7	33.1	-3.5	34	Acquisition Corr< Incorr Reversal

									Corr>Incorr
	Inferior frontal gyrus	R	9	-36.3	-7.7	29.7	-4.2	33	Acquisition Corr< Incorr Reversal Corr>Incorr
	Middle frontal gyrus	L	9	36.9	0.5	61.5	-4.4	23	Acquisition Corr< Incorr Reversal Corr>Incorr
Group x reinforcement	Inferior frontal gyrus	L	9	42.4	-4.7	34.0	3.7	27	Incorrect Ctrl>pat
3-way interaction	Inferior/middle frontal gyrus	L	47	40.1	-31.6	-1.1	4.7	22	Incorrect acq Ctrl> Pay Correct rev Ctrl > Pat
Feedback Phase									
Fixed Effects	**Region**	**L/R**	**BA**	**X**	**Y**	**Z**	**t-value**	**# voxels**	**Direction**
Reinforcement	Right Inferior, middle, superior frontal gyrus/insula Left superior, medial frontal gyrus/cingulate gyrus/anterior cingulate	R/L	47/46/9/ 10 /8/6/13/ 45/32	-28.9	-26.6	31.9	0.3	9521	Incorr > corr
	Superior, middle, inferior frontal gyrus/insula/ precentral gyrus	L	47/13/10 / 9/6/8/44/ 45	42.4	-20.9	23.2	-5.1	5020	Incorr > corr
	Inferior parietal lobe	L	40	35.3	59.5	39.3	-6.9	4994	Incorr > corr
	Superior parietal lobe	R	7	-43.0	57.8	45.3	-3.9	3083	Incorr > corr
	Inferior occipital gyrus	R	18	-51.2	54.8	-11.8	-1.0	1282	Incorr > corr
	Cuneus	R	18	-0.6	82.7	8.4	-3.2	885	Incorr > corr
	Fusiform gyrus	L	19	28.0	66.4	-28.5	-2.9	631	Incorr > corr
	Thalamus	R		0.7	5.3	9.2	-3.7	612	Incorr > corr
	Fusiform gyrus	L	19	45.5	69.3	-14.8	-3.2	296	Incorr > corr
	Lingual gyrus	R	18	-12.6	82.4	-25.2	-4.5	147	Incorr > corr
	Cingulate gyrus	L	23	1.6	30.5	28.4	-4.6	85	Incorr > corr

	Superior frontal gyrus	R	11	-21.8	-44.8	-15.0	-4.9	77	Incorr > corr
	Cingulate gyrus	L/R	23	1.2	14.1	31.1	-4.0	58	Incorr > corr
	Middle occipital gyrus	L	18	35.2	91.6	5.9	-4.3	51	Incorr > corr
	Superior frontal gyrus	L	11	23.2	-42.1	-15.3	-4.9	43	Incorr > corr
	caudate	R		-26.2	46.2	15.6	4.8	39	Corr>Incorr
	Nodule	L		-0.3	56.4	-35.6	-4.2	28	Incorr > corr
	Posterior cingulate	R		-0.1	42.5	7.6	-3.9	28	Incorr > corr
	Superior temporal gyrus	R	39	-44.2	57.7	23.2	-3.9	26	Incorr > corr
	Middle occipital gyrus	R	19	-47.9	79.5	1.3	-4.3	23	Incorr > corr
	Parahippocampal gyrus	L		8.6	38.2	4.6	-4.6	23	Incorr > corr
Phase	Inferior occipital gyrus	R	18	-37.8	84.9	-20.8	4.1	109	Acq> Rev
	Middle occipital gyrus	L	19	54.1	75.7	-1.6	4.4	48	Acq> Rev
	Cuneus	R	18	-2.5	94.9	10.2	3.8	45	Acq> Rev
Group x Phase	Superior/middle frontal gyrus	L	10	26.5	-55.7	-10.0	-4.5	34	Acquisition Ctrl>pat Reversal Ctrl< Pat
	Inferior parietal lobule	R	40	-41.9	47.5	56.6	-3.9	23	Acquisition Ctrl > Pat
Phase x Reinforcement	Inferior occipital gyrus	R	18	-25.9	90.0	-20.1	-4.3	56	Acquisition Corr< Incorr Reversal Corr>Incorr
	Fusiform gyrus	R	19	-43.8	80.4	-21.4	-4.4	40	Acquisition Corr< Incorr Reversal Corr<Incorr
	Middle occipital gyrus	R	19	-48.7	77.6	-9.8	-4.1	36	Acquisition Corr< Incorr Reversal Corr>Incorr
	Lingual gyrus	L	18	3.6	79.6	-9.6	-3.7	28	Acquisition Corr< Incorr Reversal Corr>Incorr

	Inferior parietal lobule	R	40	-57.1	57.3	45.6	-3.7	23	Acquisition Corr< Incorr Reversal Corr<Incorr
	Middle occipital gyrus	L	19	53.7	77.4	77.4	-4.2	22	Acquisition Corr< Incorr Reversal Corr>Incorr

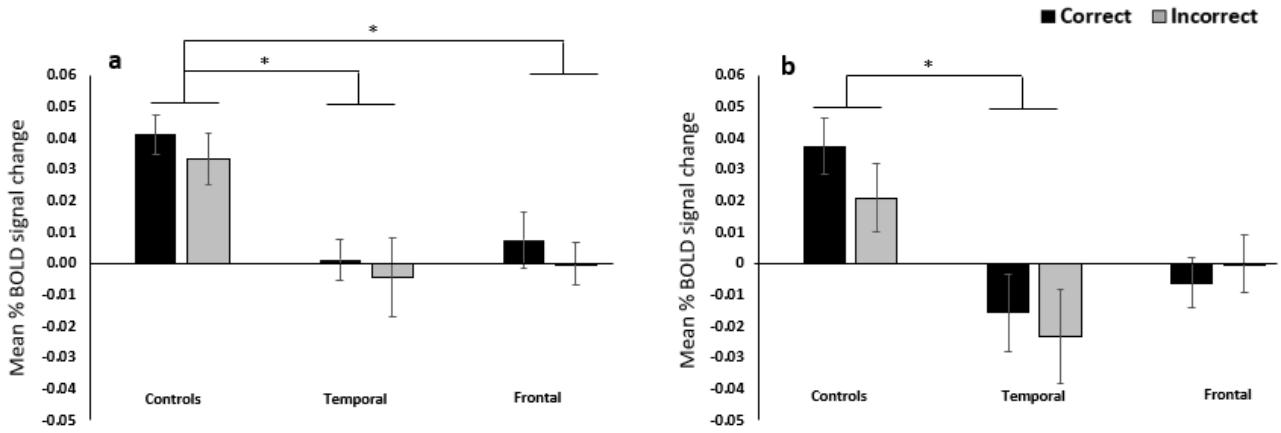
Thresholded at $p<0.001$ (t-value=3.570). All clusters survive correction for multiple comparison at $p<0.05$. Table displays regions, hemisphere (L, left; R, right), Brodmann area (BA), Montreal Neurological Institute (MNI) coordinates (x, y, z) at center of mass, maximum neural activity for the cluster (*t*-value), cluster size in voxels, and the direction of activity [Control (ctrl), Patient (pat), Acquisition (acq), Reversal (rev), Correct (corr), Incorrect (incorr)]. Focus point and regions of BA according to TT_Daemon: Talairach-Tournoux atlas. The ANOVA was completed separately for the choice and feedback phase.

4.4.4.3 Exploratory ROI Analysis

4.4.4.3.1 Choice Epoch

To examine potential differences between the atrophy groups within the resulting clusters found during the choice epoch, anatomical ROIs were created for the left vlPFC and dlPFC. A main effect of group emerged within the left vlPFC [$F(2,35)=5.9$, $p=0.006$] and left dlPFC [$F(2,35)=8.2$, $p=0.001$]. Post-hoc tests revealed that the control group exhibited greater BOLD signal relative to the frontal group (marginally for the vlPFC) and the temporal group. No differences were found between the frontal or temporal group (Figure 4.4a-b).

Figure 4.4: BOLD signal change across the control and atrophy groups during the choice epoch



Mean percent BOLD signal change during choice epoch within the (a) left dlPFC and (b) vlPFC. Error bars represent SEM. Asterisks indicate $p<0.05$. (a) Control group exhibited greater BOLD signal changes compared to the frontal and temporal group and (b) the temporal group only.

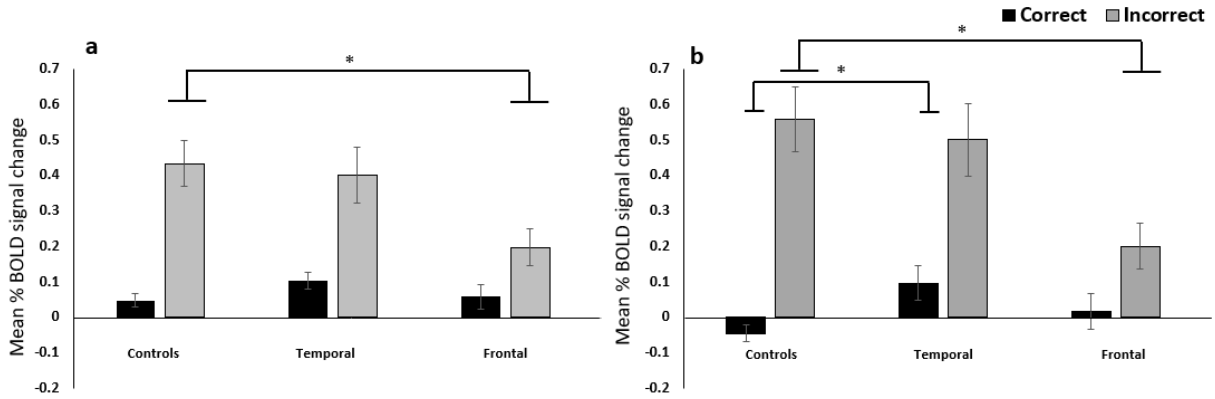
4.4.4.3.2 Feedback Epoch

Anatomical ROIs were used to partition the larger clusters found for the feedback epoch and differences between the atrophy groups and controls were assessed. A group by response accuracy interaction was observed in the right dlPFC [$F(2,35)=3.5$, $p<0.05$] and right vlPFC [$F(2,35)=3.8$, $p<0.05$]. For both regions, a one-way ANOVA for incorrect responses revealed trends (dlPFC: [$F(2,37)=2.5$, $p=0.095$]; vlPFC: [$F(2,37)=3.7$, $p=0.06$]); post-hoc tests revealed that in both regions, controls exhibited greater error-related BOLD activity relative to the frontal predominant atrophy group (dlPFC: control – frontal mean difference= 0.24, p=0.034; vlPFC: control-frontal mean difference= 0.36, p=0.02). No differences were found between the atrophy groups (dlPFC: frontal-temporal mean difference= -0.20, p=1.0; vlPFC: frontal-temporal mean difference= -0.30, p=0.08) or between controls and the temporal group (dlPFC: control-temporal mean difference= 0.03, p=0.75; vlPFC: control-temporal mean difference= 0.058, p=0.67). For

the dlPFC, no significant main effect of group was found for correct responses. Within the vlPFC a main effect of group was observed ($F(2,37)=4.1$, $p=0.025$), and an unexpected pattern emerged for correct responses in which the temporal predominant atrophy group showed enhanced activity relative to controls (Control-temporal mean difference= -0.14, $p=0.007$; Figure 4.5a-b).

As the significant group x response accuracy may have been driven by group differences between correct and incorrect responses, we computed a difference scores between the BOLD signal for correct and incorrect responses and conducted a one-way ANOVA to delineate the effect of group. For the dlPFC and vlPFC, a main effect of group emerged (dlPFC: [$F(2, 37) = 3.45$, $p=0.04$], vlPFC: [$F(2,27)= 3.78$, $p=0.03$]). For both regions, controls showed larger differences between correct and incorrect responses relative to the frontal group (dlPFC: control-frontal mean difference= -0.25, $p=0.01$; vlPFC: control-frontal mean difference = -0.42, $p=0.01$). No differences were found between controls and the temporal group (dlPFC: control-temporal mean difference: -0.08, $p=0.32$; vlPFC: mean difference= -0.20, $p=0.18$), or between the frontal and temporal groups (dlPFC: frontal-temporal mean difference= 0.16, $p=0.15$; vlPFC: mean difference=0.2, $p=0.22$).

Figure 4.5: BOLD signal change across the control and atrophy groups during the feedback epoch



Mean percent BOLD signal change during the feedback epoch within the right (a) dlPFC and (b) vlPFC during correct and incorrect trials. Error bars represent SEM. Asterisks indicate $p<0.05$. Post-hoc comparisons revealed that controls exhibited greater error-related BOLD activity relative to the frontal predominant atrophy group in the dlPFC (a) and vlPFC (b) for incorrect responses. For correct responses, patients with temporal atrophy exhibited greater activity relative to controls (b).

4.5 Discussion

Patients with FTD frequently engage in disinhibited and perseverative behaviours even when faced with negative feedback- whether social, legal or financial. Here we used reversal learning, a classic cognitive paradigm previously associated with such behaviours, to determine the functional pathophysiology leading to impaired reversal learning in patients with FTD. Reversal learning deficits have been previously shown to be correlated with behavioural problems in other populations [5]. We found that during a classic reversal learning paradigm, patients show reduced BOLD responses in the vlPFC and dlPFC. Furthermore, we also demonstrated differing BOLD responses to error feedback between patients with frontal versus temporal atrophy. These results extend the current knowledge of the underlying functional

deficits of behavioural flexibility in FTD and have implications for the selection of outcome markers and therapeutic targets for future clinical trials of symptomatic treatments.

During choice selection, patients demonstrated reduced responding within the vlPFC/insula to acquisition errors and correct reversal trials. During reversal learning, the vlPFC is implicated in selecting among competing response options. Specifically, during instances of conflict, the anterior cingulate cortex increases the representation of stimulus-motor features within the vlPFC, which, in conjunction with the caudate, flexibly controls motor responses [6]. Following this model, enhanced vlPFC activity is predicted to occur during instances of response competition including response inhibition [12]. Enhanced vlPFC has been found for correct reversals during choice selection across early relearning trials [24]. Greater response competition and response inhibition may occur during early learning trials where stimulus-response associations are still being formed relative to later learning trials where these associations are more established [24]. The functional contributions of the vlPFC is consistent with the current results demonstrating enhanced activity in healthy controls during instances of response competition to an incorrect stimulus or a previously rewarded stimulus. Overall, the reduced BOLD activity in the vlPFC in patients suggests that during response competition, deficient inputs and/or processing within the vlPFC may result in impaired flexible motor control when selecting among several potential choice options.

Impairments in response competition highlights a potential role for serotonergic modulation as a pharmacological target for reversal learning deficits in FTD. Hughes, et al. [25] showed that increasing serotonin in patients with bvFTD through the administration of an acute dose of citalopram, restored activity within the right inferior frontal gyrus as measured by MEG during response inhibition. Additionally, reducing the availability of serotonin through

tryptophan depletion significantly reduced right orbito-inferior prefrontal activity in healthy controls during an inhibitory motor control [26]. Based on the current study results and previous findings, it may be predicted that serotonin may augment neural activity within the vlPFC during response competition, which may reduce symptoms of disinhibition in patients. In fact, Herrmann, et al. [27] demonstrated that citalopram (serotonin reuptake inhibitor), was effective in treating behavioural symptoms including disinhibition, depression and irritability over a 6-week period. Further work is needed to elucidate the possible interactions between the effects of serotonin on vlPFC, response inhibition and disinhibition, and determine the implications for other disorders that feature similar behavioural disinhibition.

During errors in the choice phase, patients with FTD exhibited decreased activity relative to controls within a second region of PFC, specifically the dlPFC. Previous work has shown that within the dlPFC BOLD signal increases during instances of decision-making conflict including non-reversal errors, reversal errors and correct reversals [6,9,12]. One account suggests that during instances of decision conflict, the dmPFC/ACC engages the dlPFC to increase top-down attentional control of task-relevant features [28-31]. In these situations, increasing the salience of relevant task features may facilitate the detection of alternative cues to guide alternative behaviour [12,13]. Moreover, during response selection, the dlPFC (BA 9) demonstrates enhanced activity during early relative to late reversal trials [24]. This enhanced activity is consistent with the idea that the dlPFC is implicated in resolving decision conflict; for example, early reversal trials may place greater demands and greater conflict relative to later reversal trials wherein greater learning has taken place [24]. In patients with FTD, deficient inputs to the dlPFC, possibly from the dmPFC/ACC during instances of decision conflict (i.e. error feedback during the choice phase), may be associated with deficits in increasing attention to relevant

stimuli to resolve these conflicts and subsequently reverse a maladaptive response. Relative to healthy controls, patients with FTD are less averse to negative stimuli (e.g. unpleasant odour) and expend less effort to avoid negative stimuli [32]. Given these findings, it may be predicted that during decision-making, patients with FTD experience difficulties engaging to negatively valanced stimuli, such as error feedback, and therefore subsequent alternative motor responses are not generated. One potential intervention may include behavioural strategies that generate explicit consideration of various behavioural options and the key features to help guide optimal decision making. Another strategy may include increasing the salience of the negative information. For example, Kumfor, et al. [33] found that increasing the intensity of negative facial expressions improved emotion recognition performance in patients with bvFTD to a similar degree as controls.

As potential deficient inputs to the dlPFC, possibly from the ACC during instances of decision conflict, understanding the neurochemical mechanisms underlying conflict monitoring within the ACC have important implications in pharmacological targets for behavioural symptoms in FTD. Some evidence suggests that dopaminergic mechanisms are involved. Increased dopamine transmission has been found within the ACC during performance of a set-shifting task [34]. Additionally, elevated levels of dopamine via amphetamine administration, increases the error-related negativity (ERN) signal, an event-related potential originating within the ACC and is elicited after making an incorrect response [35]. Likewise, haloperidol (dopamine antagonist) attenuates the ERN signal [36]. Overall, these studies suggest dopaminergic involvement in the ACC during conflict monitoring and may suggest a potential avenue for pharmacological treatment in FTD. Importantly though, other research has suggested the involvement of other neurotransmitters during instances of conflict [37]. For example,

increasing the availability of norepinephrine amplifies the ERN signal during a flanker task [38]. Further work is needed to elucidate and extend the current knowledge of the roles of neurotransmitters involved in conflict monitoring within the ACC.

4.5.1 Atrophy Patterns

As the bulk of functional and lesion studies of reversal learning have implicated regions of prefrontal cortex in successful reversal learning performance, it was predicted that patients with frontal predominant atrophy would have greater neural and behavioural deficits during reversal learning relative to patients with temporal predominant atrophy and to controls. Behaviourally, patients with frontal predominant atrophy made more errors relative to healthy controls; no differences were found between patients with temporal atrophy and controls. Additionally, controls were found to exhibit greater BOLD activity relative to the frontal group for incorrect responses during the feedback stage within the dlPFC and vlPFC, while BOLD patterns were similar between controls and the temporal atrophy group.

Patients with bvFTD and SD show distinct behavioural and neuropsychological profiles; however, both frontal and temporal lobe atrophy groups experience disinhibited symptoms [39,40]. Furthermore, recent work demonstrated associations between temporal predominant atrophy and perseverative and compulsive behaviours in patients with FTD [41]. Coupled with previous studies, our findings indicate that disinhibited behaviours in temporal predominant FTD may be due to distinct cognitive mechanisms than those associated with frontal predominant FTD. For instance, it has been proposed that disinhibited behaviours in semantic dementia may in part arise from disruptions in expressive and receptive vocabulary resulting in maintaining fixed and rigid routines and thinking patterns in an effort to maintain control and understanding

during daily experiences [40,42]. Patients with temporal predominant atrophy also demonstrate disinhibition that appears distinct from language or compulsions, such as inappropriate approach and overly personal disclosures to strangers. Such behaviours may reflect deterioration of the anterior temporal lobe and its connections and with orbitofrontal cortex. In contrast, disinhibited symptoms in the frontal group likely arise from disruptions primarily within the frontal cortex [4,43]. These potentially different cognitive mechanisms underlying disinhibited symptoms in the atrophy groups illustrate the potential need for different markers to index these mechanisms and therapeutic approaches.

4.5.2 Behavioural Performance

Patients made more errors relative to controls during the acquisition and the reversal stage. Previous reversal learning studies in FTD populations included a learning criterion during the acquisition stage in which participants needed to reach prior to switching to the reversal stage [14,16,44]. In the current study no learning criterion was employed to ensure that participants had a similar number of acquisition and reversal trials for the fMRI analysis.

4.5.3 Limitations

Although all patients met the consensus criteria for probable or definite FTD, genetic or autopsy confirmation of frontotemporal dementia in this cohort has not been established for all patients; thus, patients with diseases other than FTLD may have been included in the study. Another potential limitation is the limited sample size within each of the atrophy subgroups. It is recognized that reversal learning is one of several tasks indexing behavioural flexibility; future studies utilizing other fMRI-adapted tasks are suggested in order to obtain a comprehensive

understanding of the neural deficits underlying behavioural flexibility and disinhibition in patients with FTD.

4.5.4 Conclusion

The current investigation provides insights into the regions related to inflexible decision making and responding in FTD. Patients with FTD and frontal predominant atrophy demonstrated deficits in learning and reversing stimulus-reinforcement contingencies with reduced BOLD activity in vlPFC/insula and dlPFC. Although patients with FTD with frontal predominant atrophy and temporal predominant atrophy exhibit similar caregiver-reported symptoms, our results indicate the neural mechanisms underlying these symptoms arise from different mechanisms. Future studies probing additional tasks of flexible behavioural responding and inhibition may further elucidate the neural regions and cognitive mechanisms underlying these behaviors in patients with temporal predominant atrophy and inform tailored therapeutic approaches.

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Chapter 5: General Discussion

5.1. Introduction

FTD is a neurodegenerative disorder for which there is no known cure. Clinical trials for symptomatic and disease-modifying treatments are being developed, highlighting the importance identifying disease markers to (1) indicate when treatments should be administered (2) act as outcome markers to elucidate treatment efficacy, and (3) serve as potential targets for treatments or interventions. Given the current gaps in knowledge, the objective of this thesis is to elucidate elements of the pathophysiology of FTD from the preclinical to the symptomatic disease stage. The central hypothesis is that potential markers for clinical trial designs will be identified in preclinical and symptomatic patients with FTD. The results of this thesis support the central hypothesis and demonstrate that FTD-related changes occur prior to disease onset, and that functional neural deficits indexing FTD symptoms are detectable during the symptomatic disease stage. The current thesis identifies potential markers that may be used to evaluate the clinical effects of future therapeutic interventions and to identify possible targets for future treatments.

Study I delineated the initial symptoms within each of the three most common mutation groups (*C9orf72*, *GRN*, *MAPT*) in symptomatic patients with FTD and in preclinical mutation carriers. Consistent with reports of symptomology during the course of the disease [1,2], symptomatic *MAPT* carriers frequently endorsed initial disinhibition and memory impairments, and symptomatic *GRN* carriers endorsed initial language-based symptoms most often. The second objective for study I was to examine whether preclinical mutation carriers demonstrated greater or unique behavioural/cognitive symptoms relative to biologically related non-mutation genetic carriers. At an average of 14 years *prior* to expected disease onset, preclinical *MAPT* carriers endorsed poorer mood and sleep symptoms, *C9orf72* carriers exhibited greater abnormal behaviours, and *GRN* carriers exhibited better mood relative to non-carriers. Over time, *GRN*

carriers *exhibited* poorer everyday skills relative to mutation non-carriers. These findings confirm our predictions that the initial symptoms occurring at the onset of FTD vary between and within genetic mutation groups, and that preclinical mutation carriers demonstrate aberrant behavioural/cognitive functioning prior to disease onset that is distinct from the initial symptoms that mark the start of clinical FTD.

In addition to behavioural and cognitive changes occurring during the preclinical period of FTD, study II found that preclinical mutation carriers exhibited larger ventricular volumes (i.e. greater atrophy), compared to biologically related mutation non-carriers beginning four years *prior* to expected disease onset. In contrast to the previous study's findings, no specific gene mutation patterns were found. These results provide further evidence that pathological neuroanatomical changes begin and are detectable in the preclinical period, prior to disease onset.

As disinhibition and poor behaviour flexibility is one of the earliest and debilitating symptoms in FTD, study III aimed to delineate the functional neural correlates underlying these symptoms. The current study found that during a decision-making task, patients exhibited reduced functional activity within the vlPFC and dlPFC as they selected between two choice options. These neural regions have been implicated during the initiation of motor responses to adjust current behaviour and increasing attention to task relevant stimuli [3,4]. Moreover, we also found that patients with frontal relative to temporal predominant atrophy demonstrated different patterns of neural activity relative to controls when receiving error-related feedback. These results demonstrate that patients with FTD exhibit aberrant neural functioning relative to healthy controls during a reversal learning task. Furthermore, these results also suggest that

different cognitive mechanisms may underlie disinhibition and behaviour flexibility in patients with frontal and temporal lobe atrophy patterns.

5.2 Preclinical Disease Period: The Need for Biomarkers

5.2.1 The Applicability of Biomarkers

Ideally, disease modifying treatments should be administered during the preclinical or prodromal stage of the disease, prior to the occurrence of irreversible neuronal dysfunction or loss. This highlights the needs for the identification of biomarkers during the earliest stage of the disease to evaluate the clinical effects of treatments and to indicate when treatments should be initiated [5]. Specifically, assessing at-risk individuals from families with genetic FTD is the ideal cohort to evaluate disease progression from the preclinical period to the symptomatic disease stage [6]. The results of Study I and Study II illustrate that FTD-related changes occur at the behavioural and neuroanatomical level beginning at an average of 14 years prior to expected disease onset in carriers of a pathogenic mutation. Similar to other neurodegenerative diseases [7,8], the pathophysiological mechanisms of FTD begin prior to the emergence of clinical symptoms, and thus, provides an opportunity for therapeutic treatments to intervene.

5.2.2 Preclinical Changes in Behaviour and Cognition

Recent evidence indicates that FTD-related changes in behavioural and cognition emerge and can be detected during the preclinical disease period. Relative to mutation non-carriers, *MAPT* and *GRN* preclinical carriers exhibit declines in various aspects of cognition including memory, language, and social cognition, starting eight years prior to expected disease onset [9].

As well, asymptomatic *C9orf72* carriers demonstrate impaired gestural praxis abilities 25 years prior to disease onset [10]. Although symptom characteristics have been compared across the main mutation groups in patients [1,11], gene-specific symptoms during the preclinical and prodromal disease period have not been investigated. This knowledge can potentially inform the selection of outcome measures to evaluate the effectiveness of treatments targeting specific mutations or pathologies, or basket-design trials where common symptoms arising from different pathologies are grouped together. Furthermore, previous work was limited to assessing symptoms based on aspects of cognition and broad neuropsychiatric functioning. Given these limitations, study I expanded the current knowledge by evaluating the frequency and type of symptoms that occur in genetically at-risk individuals using a comprehensive list of FTD-related symptoms across various domains (e.g. behaviour, language, cognitive, psychiatric, motor). At an average of 14 years prior to expected disease onset, *MAPT* preclinical mutation carriers endorsing worse sleep, mood and motivation, *C9orf72* carriers endorsed greater abnormal behaviours and stereotypic/motor symptoms, and *GRN* carriers endorsed better mood. Additionally, over time, *GRN* preclinical mutation carriers demonstrated worse everyday skills which involve a range of cognitive features (e.g. difficulty using electrical appliances, difficulty writing, difficulty making a hot drink, problems managing finances). The results of study I coincide with the previous literature demonstrating that disease related alterations emerge and can be detected early during the preclinical disease period, and that gene-specific pathological processes begin prior to disease onset. We expanded previous work by detecting disease-related alterations using questionnaire measures which offers a pragmatic method that can be readily employed in clinical trials to evaluate disease onset and progression. Furthermore, we illustrate behavioural and cognitive differences between the preclinical and non-mutation carriers several

years *earlier* than previously reported. Therefore, we suggest that measures assessing these gene-specific symptoms (*MAPT*: sleep, mood, motivation; *C9orf72*: abnormal behaviours, stereotypic/motor symptoms; *GRN*: mood and everyday skills), may be used as outcome measures in future clinical trials to evaluate the effectiveness of treatments.

Although we found that preclinical mutation carriers exhibited specific symptoms prior to disease onset, the frequency of the most common initial symptoms endorsed by affected patients (apathy, disinhibition, memory impairments, decreased fluency and impaired articulation), were found to be similar across preclinical mutation carriers and non-carriers. These results may suggest that *specific* symptoms may emerge just prior to or at disease onset. Similarly, during a four-year follow-up, Jiskoot, et al. [12] found that preclinical MAPT and GRN carriers who remained asymptomatic during the study period ("non-converters"), exhibited similar global cognitive functioning and neuropsychiatric features relative to non-carriers. Importantly, mutation carriers who "converted" during the follow-up period exhibited poorer cognitive and neuropsychiatric functioning relative to non-convertors and non-carriers. The results of study I raise an intriguing possibility that other symptoms are present during the preclinical period which may differ than the symptoms that emerge at disease onset. Nevertheless, these preclinical symptoms will be important to assess treatment efficacy during the preclinical period and to aid in the selection of targets for potential symptomatic treatments.

5.2.3 Preclinical Changes using Neuroimaging

Recent evidence has detected pathogenic alterations in brain structure during the asymptomatic disease stage. Investigations of genetically at-risk families have shown different cortical involvement within each of the common FTD-related mutations. For example, *MAPT*

carriers demonstrate early involvement of the anterior/medial temporal lobes; *C9orf72* carriers show a diffuse pattern of atrophy including subcortical regions of the hippocampus, thalamus and cerebellum; in *GRN* carriers, studies often report inconsistent results with only some studies finding structural changes during the preclinical disease period [13].

Ventricular volume has been proposed as a potential biomarker to index neuronal atrophy. Relative to grey matter volume, the contrasting intensity between the ventricle and surrounding tissue, makes the assessment of ventricular volume an efficient tool to be quantified with visual scales and automatic segmentation software [14]. Furthermore, the ventricles are less susceptible to scanner inhomogeneities as they located within the center of the brain and the magnet's isocenter [14,15]. Consequently, it has been proposed that ventricular volume measurements may be less variable relative to whole brain volumes and have more statistical power to detect changes over time [15]. Several studies have reported greater ventricular volume (i.e. greater neuronal loss) in patients with FTD, across the different phenotypes and genotypes, relative to healthy controls [16,17]. Although ventricular volume measurements have been considered in symptomatic patients, this measure has yet to be explored in genetically at-risk family individuals. Due to the methodological advantages, measurement of ventricular volume may offer an efficient tool to assess neurodegenerative changes during the preclinical disease period. Study II addressed this knowledge gap by examining ventricular volume in asymptomatic carriers of *C9orf72*, *GRN* or *MAPT* mutations relative to biologically related non-carriers. Critically, we found that beginning *four* years prior to expected disease onset, preclinical mutation carriers demonstrated greater ventricular volumes relative to non-carriers. Furthermore, supporting the proposed methodological advantages of ventricular volume segmentations, we found that results produced by a fully automated segmentation software were comparable to the

results completed using time-sensitive manual edits. Overall, these findings illustrate that measurements of ventricular volume are a robust tool to assess structural alterations during the preclinical disease period.

In contrast to previous literature [13], we did not find differences between genotypes in the total ventricular volume or between left and right ventricular volumes. We did not observe differences between genetic groups in the laterality index (absolute value between the left and right ventricles), which is in contrast to the predicted asymmetry atrophy pattern found in GRN mutation carriers [1]. One possibility may be that differences in neuronal loss across genotypes, particularly for GRN mutation carriers, may be detected just prior to or at disease onset. For example, Jiskoot, Panman, Meeter, Dopper, Donker Kaat, Franzen, van der Ende, van Minkelen, Rombouts, Papma and van Swieten [12] found that starting two years prior to disease onset, preclinical *MAPT* and *GRN* mutation carriers who "converted" into the symptomatic disease stage, exhibited lower grey matter volumes within regions of the frontal and temporal cortex, relative to preclinical non-convertors; structural changes were absent for preclinical carriers who remained asymptomatic during the study period.

As alterations in cortical/subcortical volumes and cortical thickness have been detected earlier than four years prior to disease onset, we suggest that assessments of grey matter volumes may be used to track disease progression and/or be used as outcome measures to assess treatment efficacy [10,18-20]. Additionally, other neuroimaging modalities including assessing white matter integrity reveal FTD-related alterations early during the preclinical disease period [6,21]. For example, Jiskoot, et al. [22] detected white matter integrity changes 30 years prior to expected disease onset in preclinical *C9orf72* and *MAPT* carriers. Additionally, studies assessing *GRN* and *MAPT* carriers detected structural and functional connectivity changes in the absence

of grey matter alterations [23,24]. As well, Feis, et al. [25] differentiated preclinical *C9orf72*, *GRN* and *MAPT* carriers from non-carriers using a multimodal MRI-based classification system that encompassed measures of radial diffusivity and white matter density. Although the authors assessed anatomical MRI, DTI and resting-state functional MRI in their classification system, the best performing classification model included only aspects of white matter integrity, supporting the notion that white matter alterations are an early marker of FTD pathology. While white matter changes have been reported earlier compared to ventricular volume changes, we suggest that given the relative ease, automaticity and reliability of ventricular volume measurements, in comparison to white matter analytic methods, direct comparisons of the sensitivity and specificity of ventricular volume to these other indices in clinical populations that may have more hetereogenous comorbidities and white matter changes would be valuable. Importantly, measures of ventricular volume, in addition to other neuroimaging modalities, may be applied as an outcome measure to assess the efficacy of disease-modifying treatments.

5.3 Symptomatic Disease Period: Identifying treatment targets

5.3.1 The Need for Treatment Targets

Currently, there are no approved disease-modifying therapies that alter the course of the disease or prevent disease progression [26]; instead, off-label use of medications that alter neurotransmission has been used for symptom management with mixed success [27]. To further advance the development of symptomatic treatment, measurements that aid in the selection of promising therapeutic targets are warranted.

5.3.2 Potential Treatment Targets for Symptomatic Disease Period

We found that across the genetic forms of FTD, the most common early symptoms were apathy, disinhibition, memory impairments, decreased fluency, and impaired articulation. Gene-specific patterns also emerged, with *C9orf72* and *MAPT* carriers endorsing disinhibition, apathy and memory symptoms most frequently, and *GRN* carriers endorsing apathy, impaired articulation and decreased fluency early during the disease. At present, there are no effective treatments for these initial symptoms in FTD. Relatedly, two key challenges in clinical trial design for FTD have been identified: (1) heterogeneity of clinical symptoms leading to difficulties in assessing treatment efficacy and (2) the rarity of FTD leading to recruitment challenges, which necessitate the need for biomarkers that can optimize treatment effects [28].

Recently, symptomatic treatment options for FTD have begun to consider the anatomical and functional neural deficits underlying FTD-related symptoms. A small number of studies have used functional imaging to delineate whether potential treatments modulate neural activity related to target symptoms. For example, based on previous work suggesting that the integrity of the inferior frontal gyrus and serotonin regulation is critical for appropriate response inhibition, Hughes, et al. [29] investigated whether increasing serotonin levels in patients with bvFTD restores neural responding within the inferior frontal gyrus during a response inhibition task. As well, it has been shown that oxytocin administered to patients with FTD modulates aberrant neural activity in brain regions related to empathy and emotional processing [30]. This work indicates potential implications for the results of Study III which assessed the neural correlates of behavioural flexibility in patients with FTD and healthy controls. Patients exhibited reduced functional activity within the vlPFC and dlPFC during the choice phase of the task, as they

selected between two different options. These neural regions have been implicated during the initiation of motor responses to adjust current behaviour and increasing attention of task relevant stimuli during error responses, respectively [3,4]. Potential interventions targeting the psychopharmacology contributions of these neural systems may be one avenue to augment neural responding within the vlPFC and dlPFC to ameliorate these symptoms in FTD. For example, increasing levels of serotonin has been found to enhance neural responding within the right inferior frontal gyrus which is associated with response inhibition [29]. We also found that patients with frontal relative to temporal predominant atrophy exhibited different neural responding when they received error-related feedback. Relative to controls, patients with frontal lobe atrophy demonstrated lower neural activity to error-feedback; no differences emerged between the control and temporal atrophy group, or between the frontal and temporal atrophy group. These results suggest that different cognitive mechanisms underlie impaired disinhibition and behavioural flexibility in the frontal and temporal patient groups, which may necessitate different therapeutic targets. Furthermore, changes in the neural activity in the vlPFC and dlPFC during reversal learning may be used as an outcome marker in proof of concept challenge studies and brief clinical trials to identify the potential of treatments targeting poor behavioural flexibility.

5.4 Limitations and Future Directions

The current thesis provides novel insights into the preclinical and symptomatic period of frontotemporal dementia that have implications for future clinical trial designs; however, certain limitations need to be addressed. Consistent with previous studies assessing preclinical changes in genetically at-risk family members of patients [31], the predicted age at disease onset for

preclinical mutation carriers was calculated using the mean age at disease onset within the participant's family for Study I and Study II. Although patient's age at disease onset is significantly correlated with the mean familial age at disease onset [31], there is a high variability in the contribution of family membership to predicted age of onset by mutation group [32]. Given these results, in study I and study II sensitivity analyses were conducted substituting the calculated age at expected disease onset for the participants current age, which yielded similar results. The continuation of longitudinal data collection in preclinical mutation carriers will be helpful to predict the age of clinical symptom occurrence. For example, Jiskoot, Panman, van Asseldonk, Franzen, Meeter, Donker Kaat, van der Ende, Dopper, Timman, van Minkelen, van Swieten, van den Berg and Papma [11] longitudinally followed preclinical mutation carriers who became symptomatic within the study window (i.e. convertors), and thus was able to determine the age in which these individuals became symptomatic. The continuation of longitudinal data collection from large FTD cohorts including GENFI, LEFTDS and ARTFL will be helpful in predicting more precise expected age at disease onset for preclinical mutation carriers and further improving the modeling of these symptoms and imaging markers.

As study I and study II examined differences in preclinical mutation carriers and biologically related non-mutation carriers, we were not able to examine the preclinical period in individuals with sporadic FTD. Examining preclinical autosomal dominant FTD gene mutation carriers provide a unique insight into the behavioural and neuroanatomical changes that lead to the conversion from the asymptomatic to the symptomatic disease stage [28]. Importantly though, the results from the genetically at-risk studies will provide new insights into the potential earliest changes in sporadic FTD [28].

Another limitation in Study II is the lack of knowledge of which genetically at-risk individuals were aware of their genetic status (carrier vs. non-carrier), which may impact their own and their informant's perspective on and reporting of cognitive and behavioural symptoms. Future studies using genetically at-risk participants should examine how one's awareness of their genetic status influences the presence and severity of FTD-related symptoms during the preclinical period.

Across all three studies, the FTD related changes in the potential markers were demonstrated at the group level; however, prior to their applicability for clinical trials, these potential markers will need to be validated at the individual level for at-risk and symptomatic patients. The continuation of multicenter research with large cohorts will be an asset for future validation at the individual level [6].

Lastly, as FTD is a heterogeneous disorder, the combination of different biomarkers may provide additive predictors of early deficits and allow accurate tracking of disease progression and severity. Future research should focus on the multimodal combination of different biomarkers (i.e. neuroimaging, behavioural and fluid markers) to further our knowledge on disease onset and monitoring disease progression and treatment response [6].

5.5 Conclusion

In conclusion, FTD is a debilitating disorder that leads to great societal, patient and caregiver burden. The heterogeneity of clinical symptoms and the rarity of FTD disorders leads to recruitment challenges and conveys the need for appropriate markers that can efficiently detect treatment effects [28]. Additionally, markers of preclinical changes may aid in the selection of potential targets for treatments and help determine when treatments (disease-

modifying or symptomatic), should be initiated. The current thesis provides novel insights into behavioural and neuroanatomical changes during the preclinical disease stage and functional neural deficits underlying behavioural difficulties during the symptomatic disease stage.

Using a large cohort of preclinical mutation carriers and biologically related non-mutation carriers, we found that mutation carriers endorsed greater symptomology during the preclinical period relative to non-carriers. Measures of these specific symptoms may be used to select treatment targets and as outcome measures to evaluate the effectiveness of treatments in future clinical trials. Furthermore, we found that preclinical mutation carriers exhibited greater ventricular volumes relative to non-carriers beginning four years prior to disease onset. Quantification of total ventricular volume may be applied in clinical trials to assess whether disease-modifying treatments slow down the progression of the disease during the preclinical stages. Lastly, we found that symptomatic patients exhibited altered neural activity within the vlPFC and dlPFC during choice selection in a decision-making task indexing behavioural flexibility. These neural changes may be used in proof of concept clinical trials to investigate symptomatic treatment effects on these altered neural regions.

Ongoing longitudinal investigations assessing symptomatic mutation carriers and genetically at-risk mutation carriers will be instrumental in increasing our understanding of the biology of FTD from the preclinical, prodromal and affected disease stages. These findings may be applied to future clinical trial designs to help assess the efficacy of treatments and to determine potential symptomatic treatment targets.

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Appendix A: Chapter 2 (Study 1) Supplementary Material

Method Section 1.0: GENFI Symptom Domains and Descriptions

Based on novel findings in the FTD literature, 31 additional symptoms, as indicated by an asterisk, were included in March 2015 (modified symptom listed below). This symptom list was based on and adapted from a consortium of validated scales including the Clinical Dementia Rating Scale (CDR [1]; FTLD-CDR [2], Social Impairment Rating Scale [3], Neuropsychiatric Inventory [4], Frontal Behavioural Inventory [5], Progressive Aphasia Severity Scale [6], Progressive Supranuclear Palsy Rating Scale [7], and Autonomic Symptoms Questionnaire (used in [8]. Further information can be gathered from the GENFI assessment manuals; see http://genfi.org.uk/.

Behaviour Symptoms

(1) Disinhibition
(2) Apathy
(3) Loss of sympathy/empathy
(4) Ritualistic/compulsive behaviour
(5) Hyperorality and appetite changes
(6) Poor response to social/emotional cues*
(7) Inappropriate trusting behaviour*

Neuropsychiatric Symptoms

(1) Visual hallucinations
(2) Auditory hallucinations
(3) Tactile hallucinations
(4) Delusions
(5) Depression
(6) Anxiety
(7) Irritability/Lability*
(8) Agitation/Aggression*
(9) Euphoria/Elation*
(10) Aberrant motor behaviour*
(11) Hypersexuality*
(12) Hyperreligiosity*
(13) Impaired sleep*
(14) Altered sense of humour*

Language Symptoms

(1) Impaired articulation
(2) Decreased fluency
(3) Impaired grammar/syntax
(4) Impaired word retrieval
(5) Impaired speech repetition
(6) Impaired sentence comprehension
(7) Impaired single word comprehension
(8) Dyslexia
(9) Dysgraphia
(10) Impaired functional communication

Cognitive Symptoms
(1) Memory impairment
(2) Impaired orientation*
(3) Impaired judgement/problem-solving
(4) Problems with community affairs*
(5) Problems at home or with hobbies*
(6) Impaired personal care*
(7) Person recognition difficulty*
(8) Impaired topographical memory*
(9) Visuo-spatial or perceptual impairment
(10) Impaired attention/concentration
(11) Bradyphrenia*

Motor Symptoms
(1) Dysarthria
(2) Dysphagia
(3) Tremor
(4) Slowness
(5) Weakness
(6) Gait disorder
(7) Falls
(8) Functional difficulties using hands*

Autonomic Symptoms
(1) Impaired blood pressure*
(2) Gastrointestinal symptoms*
(3) Impaired thermoregulation*
(4) Urinary symptoms*
(5) Altered responsiveness to pain*

Other Physical Symptoms
(1) Altered perception of sounds or music*

(2) Altered perception of smell or taste*
(3) Persistent unexplained physical symptoms*
(4) Impaired breathing*

Clinical Features
(1) Seizures
(2) Stroke or TIA
(3) Traumatic brain injury
(4) Hypertension
(5) Hypercholesterolaemia
(6) Diabetes mellitus
(7) Smoking*
(8) Excess alcohol use*
(9) Recreational drug use*
(10) Autoimmune disease*

Result Section 2.0: Analysis of symptom endorsement in symptomatic patients who completed the different versions of the GENFI symptom list

Summary of Results

As only a *single* initial symptom was selected for the symptomatic patients, we first investigated whether a different pattern of results was reported for symptomatic patients who used the original vs. modified GENFI symptom list (which included more symptom options), by evaluating the pattern of symptom endorsement at baseline in both version groups (Table B.1, B.2 and see detailed description of analysis below). Across the symptomatic cohort, the most frequent symptoms within each list were items that were present in both versions of the GENFI symptom list: disinhibition, apathy, decreased fluency, memory impairment, impaired articulation and impaired word retrieval. Thus, subsequently, data from both cohorts for the main analysis were combined.

Analysis

Of the symptomatic patients, 76 completed the original and 109 completed the modified GENFI symptom list. Disinhibition (Original: 38.8%; Modified: 4.6), apathy (Original: 28.9%; Modified: 19.3%), decreased fluency (Original: 7.9; Modified: 8.3%), impaired articulation (Original: 5.3%; Modified: 5.5%), memory impairment (Original: 5.3%; Modified: 16.5%) were the most commonly endorsed symptoms in both cohorts. Of note, 5.3% of the "original cohort" endorsed impaired word retrieval. Chi-squared tests or Fisher's exact tests were completed on each cohort to examine differences in symptom endorsement between the genetic groups.

Original Cohort: A greater proportion of *MAPT* carriers endorsed disinhibition relative to *C9orf72* and *GRN* carriers (X^2=11.1, p=0.004). Additionally, only *GRN* carriers endorsed impaired articulation (no *C9orf72* and *MAPT* carriers endorsed impaired articulation, though this contrast was only significant for *C9orf72* carriers [p=0.01, Fisher's]). No differences were found for apathy (X^2=2.2, p=0.3), decreased fluency (p=0.47, Fisher's), and memory impairment (p=0.27, Fisher's).

Modified Cohort: A greater proportion of *MAPT* carriers endorsed disinhibition (p=0.03, Fisher's) and memory impairments (p=0.04, Fisher's) more often than *C9orf72* and *GRN* carriers. Furthermore, *GRN* carriers endorsed decreased fluency more frequently relative to *C9orf72* and *MAPT* carriers (p<0.001, Fisher's). No differences were found for apathy (p=1.0, Fisher's) and impaired articulation (p=0.09, Fisher's).

Overall, the pattern of results across both cohorts were similar; both groups displayed the same predominant symptoms, and similar differences between the mutation groups. Although no

significant group differences were found for impaired articulation in the "modified cohort," both "original" and "modified" cohorts demonstrated analogous pattern of results in which *GRN* carriers showed the highest endorsement (Original: *C9orf72*=0, *GRN*=~17%, *MAPT*=0; Modified: *C9orf72*: 2%, *GRN*=12%, *MAPT*=0). Additionally, in the "modified cohort", memory impairments occurred more frequently amongst the *MAPT* carriers and *GRN* carriers endorsed decreased fluency most often. Different disease subtypes (Table A.1b) and increased samples size in the "modified cohort" (Original: N=76, Modified: N=109) and thus greater recruiting/testing sites and families, may have contributed to these slight differences. Importantly however, the inclusion of additional symptoms in the modified list did not detract reporting of symptoms found only in the original version.

Potential Limitation

Minor discrepancies in symptom endorsement reported in each version may be the result of varying sample sizes, differing proportions of FTLD sub-types, and re-categorization of symptoms from the original list into more specific symptoms in the modified list (e.g. including "poor response to social/emotional cues" and "inappropriate trusting behaviour" in the modified list may have been categorized as "disinhibition" in the original list). Importantly though, the inclusion of additional symptoms in the modified symptom list did not detract reporting of symptoms found only in the original version.

Method Section 3.0: Analysis for CBI-R change score

To improve the distribution of the residuals we attempted several statistical methods (see below). As the results of the total CBI-R change score were similar across these various techniques, we reported the results from the linear mixed model in the manuscript.

1. To improve the distribution of the residuals in the linear mixed model we included an additional fixed effect (gender) and weighted family membership. These additional predictors did not improve model fit and thus were not included in the current analysis to maintain a parsimonious model.
2. Additionally, we binned the change score into distinct categories (scores 0 or below were categorized as one group, and the remaining scores were grouped based on 20% intervals). Using these categories, we ran a general linear mixed model with multinominal distribution, and a zero inflated model with a random effect. None of these models ran successfully.
3. Using the 6 groups from above, we ran an ordinal regression (with random effects) but this model did not meet the assumption of proportionality. As well, we ran a logistic regression comparing each group to a reference group (no change or improvement in symptoms); the residuals did not improve.
4. Subsequently, we categorized the change score into two groups (group 1= participants whose symptoms deteriorated over time, group 2= participants who symptoms improved or did no change over time) and ran a general linear mixed model with a binary distribution and random effects. The residuals did not improve.

Table A.1 Symptom endorsement (%) for symptomatic patients who completed the different versions of the GENFI Symptom List

	Original GENFI symptom list				GENFI modified GENFI symptom list			
	Total (N=76)	**C9orF72 (N=34)**	**GRN (N=24)**	**MAPT (N=18)**	**Total (N=109)**	**C9orF72 (N=53)**	**GRN (N=41)**	**MAPT (N=15)**
Behavioural								
Disinhibition	36.8	35.3	16.7	66.7	4.6	1.9	2.4	20.0
Apathy	28.9	29.4	37.5	16.7	19.3	18.9	19.5	20.0
Loss of sympathy/empathy	1.3	2.9	0.0	0.0	1.8	0.0	4.9	0.0
Ritualistic/compulsive behaviour	1.3	2.9	0.0	0.0	0.9	1.9	0.0	0.0
Hyperorality and appetite changes	1.3	0.0	4.2	0.0	1.8	3.8	0.0	0.0
Poor response to social/emotional cues**					0.9	1.9	0.0	0.0
Inappropriate trusting behaviour**					0.9	1.9	0.0	0.0
Neuropsychiatric								
Visual hallucinations	1.3	2.9	0.0	0.0	0.9	1.9	0.0	0.0
Auditory hallucinations	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tactile hallucinations	0.0	0.0	0.0	0.0	0.9	1.9	0.0	0.0
Delusions	0.0	0.0	0.0	0.0	1.8	1.9	2.4	0.0
Depression	2.6	0.0	8.3	0.0	3.7	3.8	2.4	6.7
Anxiety	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Irritability/Lability**					0.9	1.9	0.0	0.0
Agitation/Aggression**					0.0	0.0	0.0	0.0
Euphoria/Elation**					0.0	0.0	0.0	0.0

	Original GENFI symptom list				GENFI modified GENFI symptom list			
	Total (N=76)	**C9orF72 (N=34)**	**GRN (N=24)**	**MAPT (N=18)**	**Total (N=109)**	**C9orF72 (N=53)**	**GRN (N=41)**	**MAPT (N=15)**
Aberrant motor behaviour**					0.0	0.0	0.0	0.0
Hypersexuality**					0.0	0.0	0.0	0.0
Hyperreligiosity**					0.0	0.0	0.0	0.0
Impaired sleep**					0.0	0.0	0.0	0.0
Altered sense of humour**					0.9	1.9	0.0	0.0
Language								
Impaired articulation	5.3	0.0	16.7	0.0	5.5	1.9	12.2	0.0
Decreased fluency	7.9	11.8	8.3	0.0	8.3	0.0	22.0	0.0
Impaired grammar/syntax	0.0	0.0	0.0	0.0	1.8	0.0	4.9	0.0
Impaired word retrieval	5.3	5.9	8.3	0.0	3.7	3.8	4.9	0.0
Impaired speech repetition	0.0	0.0	0.0	0.0	0.9	1.9	0.0	0.0
Impaired sentence comprehension	0.0	0.0	0.0	0.0	1.8	0.0	4.9	0.0
Impaired single word comprehension	0.0	0.0	0.0	0.0	0.9	1.9	0.0	0.0
Dyslexia	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Dysgraphia	0.0	0.0	0.0	0.0	1.8	1.9	2.4	0.0
Impaired functional communication	1.3	0.0	0.0	5.6	0.9	1.9	0.0	0.0
Cognitive								

	Original GENFI symptom list				GENFI modified GENFI symptom list			
	Total (N=76)	**C9orF72 (N=34)**	**GRN (N=24)**	**MAPT (N=18)**	**Total (N=109)**	**C9orF72 (N=53)**	**GRN (N=41)**	**MAPT (N=15)**
Memory Impairment	5.3	5.9	0.0	11.1	16.5	15.1	9.8	40.0
Impaired judgement/problem solving	1.3	2.9	0.0	0.0	3.7	3.8	2.4	6.7
Visuo-spatial or perceptual impairment	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Impaired attention/concentration	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Impaired Orientation**					2.8	1.9	4.9	0.0
Problems with community affairs**					0.9	1.9	0.0	0.0
Problems at home or with hobbies**					0.0	0.0	0.0	0.0
Impaired personal care**					0.0	0.0	0.0	0.0
Person recognition difficulty**					0.0	0.0	0.0	0.0
Impaired topographical memory**					0.0	0.0	0.0	0.0
Bradyphrenia **					0.0	0.0	0.0	0.0
Motor								
Dysarthria	0.0	0.0	0.0	0.0	0.9	1.9	0.0	0.0
Dysphagia	0.0	0.0	0.0	0.0	0.9	1.9	0.0	0.0
Tremor	0.0	0.0	0.0	0.0	0.9	1.9	0.0	0.0
Slowness	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Weakness	0.0	0.0	0.0	0.0	3.7	7.5	0.0	0.0
Gait disorder	0.0	0.0	0.0	0.0	1.8	3.8	0.0	0.0
Falls	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

	Original GENFI symptom list				GENFI modified GENFI symptom list			
	Total (N=76)	**C9orF72 (N=34)**	**GRN (N=24)**	**MAPT (N=18)**	**Total (N=109)**	**C9orF72 (N=53)**	**GRN (N=41)**	**MAPT (N=15)**
Functional Difficulties using hands**					2.8	3.8	0.0	6.7
Autonomic								
Impaired blood pressure**					0.0	0.0	0.0	0.0
Gastrointestinal symptoms**					0.0	0.0	0.0	0.0
Impaired thermoregulation**					0.0	0.0	0.0	0.0
Urinary symptoms**					0.0	0.0	0.0	0.0
Altered responsiveness to pain**					0.0	0.0	0.0	0.0
Other Physical								
Altered perception to sounds or music**					0.0	0.0	0.0	0.0
Altered perception of smell or taste**					0.0	0.0	0.0	0.0
Persistent unexplained physical symptoms**					0.0	0.0	0.0	0.0
Impaired breathing**					0.0	0.0	0.0	0.0
Other Disorders								
Seizures	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Stroke or TIA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Traumatic brain injury	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

	Original GENFI symptom list				GENFI modified GENFI symptom list			
	Total (N=76)	**C9orF72 (N=34)**	**GRN (N=24)**	**MAPT (N=18)**	**Total (N=109)**	**C9orF72 (N=53)**	**GRN (N=41)**	**MAPT (N=15)**
Hypertension	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hypercholesterolaemia	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Diabetes mellitus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Smoking**					0.0	0.0	0.0	0.0
Excess alcohol use**					0.0	0.0	0.0	0.0
Recreational drug use**					0.0	0.0	0.0	0.0
Autoimmune disease**					0.0	0.0	0.0	0.0

Table A.2 Demographic details for symptomatic patients completing different versions of the GENFI Symptom List

	Original Symptom List	Modified Symptom List
N	76	109
Genotype		
C9orF72	34	53
GRN	24	41
MAPT	18	15
Sex		
Female	28	49
Male	48	60
Handedness		
Right	71	103
Left	5	4
Ambidextrous	0	2
Diagnosis		
Alzheimer's Disease	1	0
ALS	0	6
Behavioural variant FTD	56	70
Corticobasal syndrome	1	2
Dementia-NOS	3	2
FTD-ALS	3	6
Other	0	2
Primary progressive aphasia	12	20
Progressive supranuclear palsy	0	1
Total number of families	68	103
Total number of sites	12	19
Age (SD)	62.9 (8.2)	61.8 (8.8)
Age of onset (SD)	58.3 (8.6)	57.9 (8.9)
Education, Yrs (SD)	12.0 (4.3)	12.4 (3.7)

ALS= Amyotrophic lateral sclerosis

Table A.3. Baseline (N=588) and Change Score (N=336) for CBI-R Total Score with age substituted for Years to Expected Symptom Onset

	Baseline[#] Estimate (95% CI)	*p*-value	Change Score Estimate (95% CI)	*p*-value
Pre-symptomatic	1.48 (0.57, 3.85)	0.42	1.76 (-0.92, 4.44)	0.2
Age	1.02 (1, 1.03)	0.02	0.046 (0.01, 0.08)	0.01
Baseline Score	-	-	-0.15 (-0.21, -0.1)	<.0001
GS*Age	0.99 (0.97, 1.02)	0.63	-0.028 (-0.08, 0.03)	0.33
Random Effects	**Estimate**	***p*-value**		
Intercept (family)	1.36	<.0001	-	-
Scale	0.32	<.0001	-	-
Residual	-	-	10.12	<.0001

- Statistics are from the Solution for Fixed Effects Table
- [#]Baseline data was modeled with a negative binomial distribution with a log link function. Estimates and confidence intervals of fixed effects are exponentiated (base e) and indicate the incident rates. Estimates below 1 indicate an inverse relationship between the variable and outcome
- GS= genetic status; CI=confidence interval; GS*age= genetic status by age interaction
- For the main effect of genetic status and GS*age interaction= reference group are the non-carriers

Table A.4. CBI-R total change score with outliers by genetic status and by genotype (N=342)

	Genotype	
	Estimate (95% CI)	***p*-value**
C9orf72	-1.98 (-4.24, 0.27)	0.08
GRN	0.39 (-1.20, 1.99)	0.63
MAPT	-0.12 (-2.5, 2.28)	0.92
YEO	0.06 (0.01, 0.11)	0.01
Baseline score	-0.16 (-0.23, -0.09)	<.0001
***C9orf72**YEO**	-0.17 (-0.28, -0.05)	0.0062
***GRN**YEO**	-0.03 (-0.12, 0.07)	0.57
***MAPT**YEO**	-0.04 (-0.18, 0.11)	0.59
Random Effects	**Estimate**	**p-value**
Family	0.51	0.30
Residual	18.7	<0.001

- Statistics are from the Solution for Fixed Effects Table
- YEO= years from expected disease onset; CI=confidence interval
- For the main effect of genotype and the genetic mutation*YEO interactions= reference group are the non-carriers

Table A.5: Symptom endorsement (%) in symptomatic patients and at-risk family members (GENFI symptom list)

	Symptomatic Patients N=185					Preclinical N=317	Non-carrier N=320	
	Total (N=185)	**C9orF72 (N=87)**	**GRN (N=65)**	**MAPT (N=33)**	**Group Contrasts**	**Symptom Endorsement**	**Symptom Endorsement**	**Group Contrasts**
Behavioural								
Disinhibition	17.8	14.9	7.7	45.5	X^2= 22.2, p<0.001 MAPT > C9orf72 & GRN	3.5	1.9	X^2= 1.6, p=0.2
Apathy	23.2	23.0	26.2	18.2	X^2= 0.8, p=0.7	4.10	4.38	X^2=0.9, p=1.0
Loss of sympathy/empathy	1.6	1.1	3.1	0.0		2.52	1.88	
Ritualistic/compulsive behaviour	1.1	2.3	0.0	0.0		1.89	1.25	
Hyperorality and appetite changes	1.6	2.3	1.5	0.0		1.26	1.25	
Poor response to social/emotional cues**	0.9	1.9	0.0	0.0		3.13	1.23	
Inappropriate trusting behaviour**	0.9	1.9	0.0	0.0		3.65	0.61	
Neuropsychiatric								
Visual hallucinations	1.1	2.3	0.0	0.0		1.89	0.00	
Auditory hallucinations	0.0	0.0	0.0	0.0		0.32	1.25	
Tactile hallucinations	0.5	1.1	0.0	0.0		0.63	0.00	
Delusions	1.1	1.1	1.5	0.0		0.32	0.94	
Depression	3.2	2.3	4.6	3.0		14.20	13.75	
Anxiety	0.0	0.0	0.0	0.0		16.09	13.13	
Irritability/Lability**	0.9	1.9	0.0	0.0		11.98	14.11	
Agitation/Aggression**	0.0	0.0	0.0	0.0		5.21	3.68	
Euphoria/Elation**	0.0	0.0	0.0	0.0		2.60	0.61	
Aberrant motor behaviour**	0.0	0.0	0.0	0.0		3.13	0.61	
Hypersexuality**	0.0	0.0	0.0	0.0		0.52	0.0	
Hyperreligiosity**	0.0	0.0	0.0	0.0		1.04	0.0	
Impaired sleep**	0.0	0.0	0.0	0.0		14.58	12.27	
Altered sense of humour**	0.9	1.9	0.0	0.0		2.60	1.23	
Language								
Impaired articulation	5.4	1.1	13.8	0.0	p=0.001$^{*\#}$ GRN > C9orf72 & MAPT	1.58	1.88	X^2= 0.08, p=0.77
Decreased fluency	8.1	4.6	16.9	0.0	p=0.005$^{*\#}$ GRN > C9orf72 & MAPT	2.52	3.13	X^2=0.21, p=0.65
Impaired grammar/syntax	1.1	0.0	3.1	0.0		0.95	1.25	

Impaired word retrieval	4.3	4.6	6.2	0.0		7.26	10.63	
Impaired speech repetition	0.5	1.1	0.0	0.0		0.00	0.31	
Impaired sentence comprehension	1.1	0.0	3.1	0.0		0.95	0.31	
Impaired single word comprehension	0.5	1.1	0.0	0.0		0.95	0.31	
Dyslexia	0.0	0.0	0.0	0.0		1.89	1.56	
Dysgraphia	1.1	1.1	1.5	0.0		1.26	2.50	
Impaired functional communication	1.1	1.1	0.0	3.0		0.63	0.31	
Cognitive								
Memory Impairment	11.9	11.5	6.2	24.2	p=0.46$^{*\#}$	10.41	12.50	X^2= 0.69, p= 0.41
Impaired judgement/problem solving	2.7	3.4	1.5	3.0		1.58	1.56	
Visuo-spatial or perceptual impairment	0.0	0.0	0.0	0.0		0.95	0.31	
Impaired attention/concentration	0.0	0.0	0.0	0.0		5.99	8.75	
Impaired Orientation**	2.8	1.9	4.9	0.0		2.08	0.0	
Problems with community affairs**	0.9	1.9	0.0	0.0		1.04	0.6	
Problems at home or with hobbies**	0.0	0.0	0.0	0.0		1.04	1.23	
Impaired personal care**	0.0	0.0	0.0	0.0		0.52	0.0	
Person recognition difficulty**	0.0	0.0	0.0	0.0		1.04	3.07	
Impaired topographical memory**	0.0	0.0	0.0	0.0		2.60	2.45	
Bradyphrenia**	0.0	0.0	0.0	0.0		2.60	3.68	
Motor								
Dysarthria	0.5	1.1	0.0	0.0		0.63	0.94	
Dysphagia	0.5	1.1	0.0	0.0		1.26	0.94	
Tremor	0.5	1.1	0.0	0.0		2.21	5.63	
Slowness	0.0	0.0	0.0	0.0		0.32	1.56	
Weakness	2.2	4.6	0.0	0.0		0.63	0.00	
Gait disorder	1.1	2.3	0.0	0.0		0.32	0.94	
Falls	0.0	0.0	0.0	0.0		0.00	0.63	
Functional Difficulties using hands**	2.8	3.8	0.0	6.7		1.0	0.0	
Autonomic								
Impaired blood pressure**	0.0	0.0	0.0	0.0		5.73	4.29	
Gastrointestinal symptoms**	0.0	0.0	0.0	0.0		2.60	5.52	
Impaired thermoregulation**	0.0	0.0	0.0	0.0		4.17	5.52	
Urinary symptoms**	0.0	0.0	0.0	0.0		4.69	4.29	

Altered responsiveness to pain**	0.0	0.0	0.0	0.0		1.04	1.84	
Other Physical								
Altered perception to sounds or music**	0.0	0.0	0.0	0.0		0.52	1.84	
Altered perception of smell or taste**	0.0	0.0	0.0	0.0		2.1	2.5	
Persistent unexplained physical symptoms**	0.0	0.0	0.0	0.0		2.1	0.0	
Impaired breathing**	0.0	0.0	0.0	0.0		0.5	1.2	
Clinical Features								
Seizures	0.0	0.0	0.0	0.0		1.58	0.94	
Stroke or TIA	0.0	0.0	0.0	0.0		0.32	0.63	
Traumatic brain injury	0.0	0.0	0.0	0.0		9.46	11.56	
Hypertension	0.0	0.0	0.0	0.0		12.62	11.56	
Hypercholesterolaemia	0.0	0.0	0.0	0.0		9.78	11.56	
Diabetes mellitus	0.0	0.0	0.0	0.0		2.21	2.19	
Smoking**	0.0	0.0	0.0	0.0		27.08	34.97	
Excess alcohol use**	0.0	0.0	0.0	0.0		4.69	4.91	
Recreational drug use**	0.0	0.0	0.0	0.0		9.38	11.0	
Autoimmune disease**	0.0	0.0	0.0	0.0		5.73	6.75	

**Indicates sub-symptoms collected using the modified GENFI symptom list (Symptomatic: N=109; Preclinical=192, Non-carriers N=163)

*#Fisher's Exact Test was used as the expected count was less than 5

Table A.6. Initial symptoms of symptomatic patients from the same family

Number of participants within each family	Gene	First symptoms reported	Congruency Score (%)
2	*GRN*	apathy (n=1), fluency (n=1)	0
2	*GRN*	apathy (n=1) & fluency (n=1)	0
2	*GRN*	apathy (n=1) & articulation (n=1)	0
3	*GRN*	apathy (n=2) & memory impairment (n=1)	33
5	*GRN*	apathy (n=1) & hyperorality and appetite change (n=1), depression (n=1) & articulation (n=2)	10
3	*C9orf72*	disinhibition (n=1) & depression (n=1) & tremor (n=1)	0
2	*C9orf72*	apathy (n=1) & fluency (n=1)	0
2	*C9orf72*	disinhibition (n=1) & memory impairment (n=1)	0
2	*C9orf72*	depression (n=1) & memory impairment (n=1)	0
2	*MAPT*	apathy (n=1) & memory impairment (n=1)	0
2	*MAPT*	disinhibition (n=2)	100
2	*MAPT*	memory (n=2)	100
3	*MAPT*	apathy (n=2) & impaired judgement/problem-solving (n=1)	33
3	*MAPT*	disinhibition (n=2) & depression (n=1)	33

The average congruency score across the cohort was 19%. This was calculated as the number of congruent combinations divided by the number of possible pairwise combinations

Table A.7. Initial symptoms of symptomatic patients with the same specific genotype

Gene	Gene Type	Number of participants	First Symptom Reported	Congruency Score (%)
MAPT	Q351R	2	memory impairment (n=2)	100
MAPT	G272V	3	disinhibition (n=2), depression (n=1)	33
MAPT	P301L	7	disinhibition (n=3), apathy (n=3), impaired judgement/problem solving (n=1)	29
MAPT	R406W	7	disinhibition (n=3), apathy (n=1), memory impairment (n=3)	29
MAPT	IVS10+16	9	disinhibition (n=5), apathy (n=1), memory impairment (n=3)	36
GRN	C149fs	2	Disinhibition (n=1), impaired articulation (n=1)	0
GRN	G35fs	2	Apathy (n=1), decreased fluency (n=1)	0
GRN	Q130fs (388_391delCAGT)	2	Apathy (n=1), impaired grammar/syntax (n=1)	0
GRN	C31fs	4	Apathy (n=1), loss of sympathy/empathy (n=1), impaired articulation (n=1), decreased fluency (n=1)	0
GRN	S82fs	5	Apathy (n=1), hyperorality and appetite changes (n=1), depression (n=1), impaired articulation (n=2)	10
GRN	IVS7-1G>A	8	Apathy (n=2), loss of sympathy/empathy (n=1), decreased fluency (n=1), impaired word retrieval (n=1), impaired sentence completion (n=1), memory impairment (n=2)	7
GRN	T272fs	24	Disinhibition (n=1), apathy (n=10), impaired articulation (n=5), decreased fluency (n=4), impaired grammar/syntax (n=1), impaired word retrieval (n=1), dysgraphia (n=1), impaired judgement/problem solving (n=1)	22

The average congruency score was 33% for *MAPT* and 20 for *GRN*. This was calculated as the number of congruent combinations divided by the number of possible pairwise combinations

Table A.8. Baseline symptom endorsement on the GENFI symptom list (%) by gene mutation type in at-risk[t] family members

	C9orf72			*GRN*			*MAPT*		
	Preclinical (n=117)	**Non-carrier (n=115)**	**Contrast (test statistic, *p*-value)**	**Preclinical (n=144)**	**Non-carrier (n=144)**	**Contrast (test statistic, *p*-value)**	**Preclinical (n=56)**	**Non-carrier (n=61)**	**Contrast (test statistic, *p*-value)**
Sub-symptoms*									
Disinhibition	6.0	1.7	0.17[#]	2.1	2.1	1.00[#]	1.8	1.6	1.00[#]
Apathy	6.8	6.1	X^2=0.05, *p*=0.82	2.8	3.5	1.00[#]	1.8	3.3	1.00[#]
Decreased fluency	1.7	6.1	0.10[#]	2.8	0.7	0.37[#]	3.6	3.3	1.00[#]
Impaired articulation	1.7	0.9	1.00[#]	1.4	3.5	0.44[#]	1.8	0	0.48[#]
Memory impairment	13.7	13.9	X^2=0.002, *p*=0.96	8.3	11.8	X^2=0.96, *p*=0.33	8.9	11.5	X^2=0.21, *p*=0.65

- *Reflects the sub-symptoms that were most frequently endorsed as "first symptoms" by symptomatic patients
- [#] Fisher's Exact Test was used as the expected count was less than 5
- [t] At-risk: preclinical carriers and non-carriers
- No differences were found between the preclinical mutation groups (Disinhibition: Fisher's Exact Test p=0.21; Apathy: Fisher's Exact Test, p=0.23; Memory X^2=2.14, p=0.4; Fluency: Fisher's Exact Test p=0.64; Articulation : Fisher's Exact Test p=1.0)

Table A.9. Symptom endorsement (%) between the first and final visit for at-risk individuals (GENFI symptom list)

	Pre-symptomatic Mutation Carriers						Non-carriers							
	Total (N=196)	**Mean time interval, Yrs (SD)**	**Mean YEO (SD)[#]**	***C9orf72 (n=58)***	***GRN (n=95)***	***MAPT (n=43)***	**Total (N=202)**	**Mean time interval, Yrs (SD)**	**Mean YEO (SD)[#]**	***C9orf72 (n=62)***	***GRN (n=103)***	***MAPT (n=37)***	**Group Contrasts**	**Genotype Contrasts**
Disinhibition														
No change	96.9	2.6 (1.3) Min: 0.8 Max: 5.6	-14.1 (11.2)	98.3	96.8	95.3	98.0	2.5 (1.5) Min: 0.8 Max: 5.6	-11.6 (13.3)	98.4	97.1	100	*p*=0.8	*C9orf72*: *p*=1.0
Increase symptom endorsement	2.0	2.4 (1.7) Min: 0.9 Max: 4.5	-7.4 (17.1)	1.7	2.1	2.3	1.0	3.3 (1.7) Min: 2.1 Max: 4.5	-6.4 (21.4)	1.6	1.0	0.0		*GRN:* *p*=0.847
Decrease in symptom endorsement	1.0	3.4 (2.8) Min: 1.4 Max: 5.4	-0.8 (14.3)	0.0	1.0	2.3	1.0	3.0 (2.7) Min: 1.1 Max: 4.9	-19.0 (1.0)	0.0	1.9	0.0		*MAPT*: *p*=1.0
Apathy														
No change	95.4	2.6 (1.3) Min: 0.8 Max: 5.6	-14.2 (11.2)	94.8	95.8	95.3	95.5	2.5 (1.4) Min: 0.8 Max: 5.6	-11.7 (13.2)	95.2	96.1	94.6	*p*= 0.9	*C9orf72*: *p*=1.0
Increase symptom endorsement	2.0	3.4 (1.8) Min: 1.7 Max: 5.2	-1.0 (11.9)	0.0	3.2	2.3	2.5	3.5 (2.3) Min: 1.0 Max: 5.6	0.6 (13.46)	0.0	2.9	5.4		*GRN:* *p*=1.0
Decrease in symptom endorsement	2.6	3.1 (1.8) Min: 1.1 Max: 5.0	-9.2 (12.5)	5.2	1.1	2.3	2.0	2.1 (1.9) Min: 1.0 Max: 4.9	-21.9 (3.4)	4.8	1.0	0.0		*MAPT*: *p*=0.8
Decreased fluency														
No change	96.4	2.6 (1.4) Min: 0.8 Max: 5.6	-13.9 (11.4)	98.3	97.9	90.7	96.5	2.5 (1.5) Min: 0.78 Max: 5.6	-11.9 (13.3)	95.2	96.1	100	*p*=0.9	*C9orf72*: *p*=0.746319
Increase symptom endorsement	2.6	1.8 (1.0) Min: 0.9 Max: 3.3	-17.7 (7.0)	1.7	2.1	4.7	2.0	1.0 (0.1) Min: 1.0 Max: 1.1	-1.4 (10.3)	1.6	2.9	0.0		*GRN:* *p*=1.0
Decrease in symptom endorsement	1.0	2.2 (1.2) Min: 1.4 Max: 3.1	0.2 (12.8)	0.0	0.0	4.7	1.5	3.2 (2.0) Min: 1.0 Max: 4.9	-7.4 (10.2)	3.2	1.0	0.0		*MAPT*: *p*=0.247
Memory impairments														
No change	85.7	2.6(1.3) Min: 0.9 Max: 5.4	-14.0 (11.0)	86.2	87.4	81.4	85.1	2.5 (1.5) Min: 0.8 Max: 5.6	-12.6 (12.5)	82.3	85.4	89.2	X^2=0.7, *p*=0.7	*C9orf72*: *p*=0.5

	Pre-symptomatic Mutation Carriers						Non-carriers							
	Total (N=196)	**Mean time interval, Yrs (SD)**	**Mean YEO (SD)[#]**	***C9orf72 (n=58)***	***GRN (n=95)***	***MAPT (n=43)***	**Total (N=202)**	**Mean time interval, Yrs (SD)**	**Mean YEO (SD)[#]**	***C9orf72 (n=62)***	***GRN (n=103)***	***MAPT (n=37)***	**Group Contrasts**	**Genotype Contrasts**
Increase symptom endorsement	8.7	3.0 (1.8) Min: 0.8 Max: 5.56	-11.6 (14.2)	10.3	6.3	11.6	7.4	2.7 (1.8) Min: 0.9 Max: 5.6	-4.2 (14.8)	8.1	6.8	8.1		*GRN:* X^2=0.2, *p*=0.9 *MAPT*: *p*=0.7
Decrease in symptom endorsement	5.6	3.0 (2.0) Min: 1.0 Max: 5.5	-13.9 (13.1)	3.4	6.3	7.0	7.4	2.1 (1.5) Min: 1.0 Max: 5.3	-7.9 (17.3)	9.7	7.8	2.7		
Articulation Impairments														
No change	96.9	2.6 (1.4) Min: 0.8 Max: 5.6	-14.0 (11.3)	98.3	95.8	97.7	96.5	2.5 (1.5) Min: 0.8 Max: 5.6	-11.5 (13.2)	100	93.2	100	*p*= 0.7	*C9orf72*: *p*=0.483 *GRN:* *p*=0.791 *MAPT*: *p*=1.0
Increase symptom endorsement	2.6	2.8 (1.7) Min: 1.0 Max: 5.5	-5.8 (13.9)	1.7	3.2	2.3	2.0	2.8 (2.1) Min: 1.0 Max: 4.9	-4.6 (14.7)	0.0	3.9	0.0		
Decrease in symptom endorsement	0.5	--	--	0.0	1.1	0.0	1.5	1.3 (0.4) Min: 1. Max: 1.7	-27.3 (5.4)	0.0	2.9	0.0		

- Number of participants for each maximum visit: Maximum of 2 visits: N=178 (n=80 pre-symptomatic; n=98 non-carrier); Maximum of 3 visits: N=130 (n=72 pre-symptomatic; 58 non-carriers); Maximum of 4 visits: N=57 (n=30 pre-symptomatic; 27 non-carriers); Maximum of 5 visits: N=25 (n=10 pre-symptomatic; n=15 non-carriers); Maximum of 6 visits: N= 8 (n=4 pre-symptomatic; n=4 non-carriers)
- *Sub-symptoms are coded as 0=no change in symptom endorsement, 1=increase in symptom endorsement, -1 decrease in symptom endorsement
- [#]YEO=Years from expected symptom onset. Values represent estimates from the initial visit. Mean YEO is only reported for categories where n>1 to prevent disclosure of genetic status

Table A.10: Sensitivity and Specificity Scores (%) for Gene Composite Indices

	C9orf72/MAPT Composite Index		GRN Composite Index	
Gene Group	**Sensitivity (95% CI)**	**Specificity (95% CI)**	**Sensitivity (95% CI)**	**Specificity (95% CI)**
[*]Symptomatic vs. Non-carrier				
C9orf72	96.4 (89.9-99.3)	80.4 (71.4-87.6)	89.3 (80.6-95.0)	91.2 (83.9-95.9)
GRN	96.6 (88.1-99.6)	80.4 (71.4-87.6)	98.3 (90.8-100.0)	91.2 (83.9-95.9)
MAPT	93.6 (78.6-99.2)	80.4 (71.4-87.6)	80.7 (62.5-92.6)	91.2 (83.9-95.9)
[**]Preclinical vs. Non-carriers (Beginning -5 years of expected disease onset)				
C9orf72	20.0 (6.8 - 40.7)	80.4 (71.4 - 87.6)	12.0 (2.6 - 31.2)	91.2 (83.9 - 95.9)
GRN	14.3 (4.8 - 30.3)	80.4 (71.4 - 87.6)	8.6 (1.8 - 23.1)	91.2 (83.9 - 95.9)
MAPT	18.2 (2.3 - 51.8)	80.4 (71.4 - 87.6)	9.1 (0.2 - 41.3)	91.2 (83.9 - 95.9)
[^]Preclinical vs. Non-carriers (Beginning -2 years of expected disease onset)				
C9orf72	20.0 (4.3 - 48.1)	78.4 (67.3 - 87.1)	13.3 (1.7 - 40.5)	90.5 (81.5 - 96.1)
GRN	15.4 (4.4 - 34.9)	78.4 (67.3 - 87.1)	11.5 (2.5 - 30.2)	90.5 (81.5 - 96.1)
MAPT	28.6 (3.7 - 71.0)	78.4 (67.3 - 87.1)	14.3 (0.4 - 57.9)	90.5 (81.5 - 96.1)
[^^]Preclinical vs. Non-carriers (Beginning 0 years of expected disease onset)				
C9orf72	23.1 (5.0 - 53.8)	76.2 (63.8 - 86.0)	15.4 (1.9 - 45.5)	90.5 (80.4 - 96.4)
GRN	22.2 (6.4 - 47.6)	76.2 (63.8 - 86.0)	16.7 (3.6 - 41.4)	90.5 (80.4 - 96.4)
MAPT	33.3 (4.3 - 77.7)	76.2 (63.8 - 86.0)	16.7 (0.4-64.1)	90.5 (80.4 - 96.4)

[*]As symptomatic carriers were older than non-carriers the following comparison only includes non-carriers who were at least -5 years from disease onset. Symptomatic carriers: *C9orf92*: n=84, *GRN*: n=58, *MAPT*: n=31; *Non-carriers* n=102

[**]Preclinical: *C9orf92*: n=25, *GRN*: n=35, *MAPT*: n=11; *Non-carriers* n=102

[^]Preclinical: *C9orf92*: n=15, *GRN*: n=26, *MAPT*: n=7; *Non-carriers* n=74

[^^]Preclinical: *C9orf92*: n=13, *GRN*: n=18, *MAPT*: n=6; *Non-carriers* n=63

CI= 95% confidence intervals

Table A.11: Mean (SD) Composite Scores for At-Risk Groups

	C9orf72/MAPT Composite Index		GRN Composite Index	
	Preclinical	**Non-carrier**	**Preclinical**	**Non-carrier**
At-risk from -5 years to expected onset				
C9orf72	0.2 (0.5)	0.3 (0.6)	0.2 (0.5)	0.1 (0.5)
GRN	0.2 (0.5)		0.1 (0.6)	
MAPT	0.3 (0.6)		0.2 (0.6)	
At-risk from -2 years to expected onset				
C9orf72	0.3 (0.6)	0.3 (0.7)	0.2 (0.6)	0.1 (0.5)
GRN	0.2 (0.5)		0.2 (0.36)	
MAPT	0.4 (0.8)		0.3 (0.8)	
At-risk from -0 years to expected onset				
C9orf72	0.3 (0.6)	0.3 (0.7)	0.2 (0.6)	0.1 (0.5)
GRN	0.3 (0.6)		0.3 (0.8)	
MAPT	0.5 (0.8)		0.3 (0.8)	

Only one symptom was selected as the initial symptom for affected patients

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Appendix B: Chapter 3 (Study 2) Supplementary Material

Results Section 1.0: Additional analysis with all genetic mutation carriers relative to non-mutation carriers

Participants

A total of 127 participants met the inclusion criteria. After processing in FreeSurfer, 4 participants were removed prior to statistical analysis: 1 due to scaling errors, 1 with extensive segmentation errors, and 2 found to be extreme outliers (1 carrier and 1 non-carrier from *PGRN* families; mean volumes >3 SD), leaving 123 participants from 56 family cohorts, entered into the statistical model (Table A.1).

Carriers vs. Non-carriers

In the comparison of all carriers (symptomatic and preclinical) relative to non-carriers, the final model included a genetic status by time to disease onset (linear) interaction (Table A.2). Due to the significant correlation between age and expected years to disease onset, ($r=0.91$, $p<0.0001$), age could not be included in the model due to multi-collinearity. There was a significant genetic status by time to disease onset (linear) interaction. In a sensitivity analysis with one potentially highly-influential participant removed (carrier), the significance of the main effects and interaction did not change and thus their data remained in the analysis. Additionally, visual inspection of the scatterplots demonstrated an extreme case (non-carrier). Unadjusted post-hoc *t*-tests of the genetic status by time to disease onset interaction demonstrated greater ventricular volume in carriers relative to non-carriers starting at 10 years prior to expected disease onset, or

at 12 years prior to expected disease onset when one extreme case (non-carrier) was removed from the model (Figure A.1, Table A.3).

Years from Actual Disease onset: The above analysis used calculated years to *expected* disease onset for all participants as in the prior GENFI study. To confirm that the results were similar when the age of disease onset was used for symptomatic carriers, the above analysis was repeated using actual time from disease onset, calculated by subtracting the age at disease onset from the participant's current age at the time of the baseline and follow-up scans. The above analysis was repeated using the final model with the same extreme case removed, substituting actual time from disease onset for all symptomatic carriers and for three preclinical carriers who became symptomatic during the 1year interval between baseline and follow up. Similar to the previous results, there was a significant genetic status by years to onset interaction, where carriers showed greater volumes beginning 10 years prior to disease onset relative to non-carriers (Table A.4).

Manually Edited Versus Fully Automated Ventricular Volumes

Manual edits to the ventricular segmentations performed in FreeSurfer were made on all study participants for each time point (mean differences in edited vs. unedited volumes are reported in supplementary analysis). Substitution of the fully automated ventricular volumes produced by FreeSurfer into the final models resulted in similar findings, demonstrating that for all carriers vs. non-carriers, significant differences were observed at 12 years prior to expected disease onset (Tables A.5a-b). See Table A.6 for annualized change of unedited ventricular volume.

Total Ventricular Expansion over 1 year

To assess potential differences in ventricular expansion over the 1-year interval, an additional model was comprised that included the same family and participant random effects as above and the following fixed effects: visit, years to disease onset (linear and quadratic terms), genetic status and an interaction between visit and genetic status. A sensitivity analysis for all carriers vs. non-carriers identified one high-influential participant (carrier) but their removal did not change the significance of the main effects and interaction and thus, they were retained. Carriers as a whole demonstrated greater ventricular volume at baseline (p=0.03) and follow-up (p=0.003) relative to non-carriers. Additionally, carriers showed significant ventricular expansion between baseline and follow up (p<0.001), whereas non-carriers did not (p=0.23). Annual rate of change of total ventricular volume is presented in Table A.7, as a function of genetic status and years to expected disease onset.

Mutation Type

Specifically, utilizing the final models from previous analysis (with the extreme case removed for carriers vs. non-carriers comparison), we included, genotype (*C9orf72, PGRN, MAPT*) and the interaction between genotype and genetic status as fixed effects in the model. Significant interactions were decomposed using simple effects estimation. For total ventricular volume, for all carriers vs. non-carriers, the interaction between genotype and genetic status did not reach significance (p=0.10). Additionally, there was no significant interaction between genetic status and genotype for the laterality index (p=0.18).

Table B.1: Demographic characteristics of study participants (N=123)

	Symptomatic (n=21)	Preclinical Carrier (n=46)	Non-Carriers (n=56)	All Carriers vs. Non-carriers	Preclinical carriers vs. Non-carriers
Genotype				*p*=0.18	*p*=0.74
C9orf72	13	13	13		
PGRN	5	29	36		
MAPT	3	4	7		
Sex				*p*=0.27	*p*=0.52
Female	9	25	34		
Male	12	21	22		
Years from expected disease onset at baseline Mean (SD)	5.16 (5.0)	-13.34 (12.65)	-9.14 (15.60)	*p*=0.55	*p*=0.14
Age (SD)	64.37 (6.19)	44.66 (11.14)	50.56 (15.64)	*p*=0.92	*p*=0.03*
Years of education (SD)	13.09 (4.11)	14.20 (3.47)	14.34 (3.51)	*p*=0.46	*p*=0.84

Group differences were assessed using chi-square tests and *t*-tests. *****significant at $p<0.05$
Symptomatic carriers (n=18) include 3 progressors

Table B.2: Estimates for total ventricular volume of carriers (n=67) and non-carriers (n=55) with no extreme case (n=1)

Fixed Effects	Estimate	SE	*p*-value	CI (95%)
Intercept	-0.70	0.56	0.22	-1.83, 0.43
Visit (ref=follow-up)	-0.04	0.02	0.07	-0.08, 0.003
GS (ref=non-carriers)	0.70	0.17	<.0001*	0.37, 1.04
Time from disease onset	-0.06	0.02	0.006*	-0.10, -0.02

Time from disease onset^2	0.001	0.0003	<.0001*	0.0008, 0.002
Time from disease onset*GS	0.03	0.01	0.001*	0.01, 0.05
Random Effects	**Estimate**	**SE**	***p*-value**	
Family Membership	0.18	0.17	0.13	
Participant	0.58	0.13	<.0001*	
Residual	0.02	0.003	<.0001*	

SE=standard error; GS=Genetic Status (carrier vs. non-carrier); CI=Confidence interval; *****significant at $p<0.05$

Table B.3. Estimated difference in ventricle volume between mutation carriers (n=67) and non-carriers (n=55) by time from expected disease onset

	-25 years	-20 years	-15 years	-10 years	-5 years	0 years	5 years	10 years
Total Ventricle$^+$								
Estimate	-0.12	0.06	0.22	0.38	0.54	0.70	0.86	1.02
SE	0.22	0.19	0.17	0.15	0.15	0.17	0.20	0.23
p-value	0.64	0.7699	0.1929	0.0144*	0.0006*	<.0001*	<.0001*	<.0001*
Total Left Ventricle^{++}								
Estimate	-0.06	0.02	0.11	0.19	0.27	0.36	0.44	0.52
SE	0.11	0.09	0.08	0.07	0.07	0.08	0.09	0.11
p-value	0.57	0.79	0.17	0.01*	0.0003*	<.0001*	<.0001*	<.0001*
Total Right Ventricle^{++}								
Estimate	-0.06	0.01	0.08	0.15	0.21	0.28	0.35	0.42
SE	0.12	0.10	0.09	0.08	0.08	0.09	0.11	0.12
p-value	0.65	0.91	0.38	0.08	0.01*	0.003*	0.001*	0.001*
Third Ventricle								
Estimate	0.002	0.007	0.01	0.02	0.02	0.03	0.04	0.04
SE	0.006	0.006	0.005	0.004	0.004	0.005	0.006	0.007
p-value	0.79	0.18	0.007*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*

SE=standard error**; ***significant at $p<0.05$

+ Total volume is comprised of the left and right lateral, inferior, and third and fourth ventricles

++ Total left and right ventricles are composed of the lateral and inferior ventricles

Table B.4: Estimates for total ventricular volume using actual years to disease onset for carriers. Using final model with no extreme case (N=122)

Fixed Effects	**Estimate**	**SE**	***p*-value**	**CI (95%)**
Intercept	-0.03	0.56	0.95	-1.15, 1.08
Visit (ref=follow-up)	-0.04	0.02	0.04*	-0.08, -0.003
GS (ref=non-carriers)	0.60	0.18	0.0009*	0.25, 0.95
Real time from disease onset	-0.04	0.02	0.08*	-0.08, 0.005
Real time from disease onset^2	0.001	0.0003	0.0005*	0.0005, 0.002
Time from disease onset*GS	0.03	0.01	0.010*	0.006, 0.05
Random Effects	**Estimate**	**SE**	***p*-Value**	
Family Membership	0.12	0.17	0.23	
Participant	0.68	0.15	<.0001*	
Residual	0.02	0.003	<.0001*	

SE=standard error; GS=Genetic Status (carrier vs. non-carrier); *****significant at $p<0.05$

Table B.5a: Estimates for carriers (n=67) and non-carriers (n=55) for *unedited* ventricular volumes with no extreme case (n=1)

Fixed Effects	**Estimate**	**SE**	***p*-value**	**CI (95%)**
Intercept	-0.72	0.55	0.19	-1.83, 0.38
Visit (ref=follow-up)	-0.04	0.02	0.06	-0.08, 0.002
GS (ref=non-carrier)	0.72	0.17	<.0001*	0.39, 1.06
Time from disease onset	-0.06	0.02	0.004*	-0.10, -0.02
Time from disease onset^2	0.001	0.0003	<.0001*	0.0009, 0.002
Time from disease onset*GS	0.03	0.009	0.0007*	0.01, 0.05
Random Effects	**Estimate**	**SE**	***p*-Value**	
Family Membership	0.19	0.17	0.13	
Participant	0.58	0.13	<.0001*	
Residual	0.02	0.003	<.0001*	

SE=standard error; GS=Genetic Status (carrier vs. non-carrier); *****significant at $p<0.05$

Table B.5b: Estimates between carriers (n=67) and non-carriers (n=55) for *unedited* total ventricular volume by time to expected disease onset

	-25 years	-20 years	-15 years	-10 years	-5 years	0 years	5 years	10 years
Estimate	-0.12	0.05	0.22	0.39	0.56	0.72	0.89	1.06
SE	0.22	0.19	0.17	0.15	0.15	0.17	0.20	0.23
***p*-value**	0.61	0.78	0.19	0.01*	0.0004*	<.0001*	<.0001*	<.0001*

SE=standard error; *****significant at $p<0.05$

Table B.6. Annual percent change of *unedited* total ventricular volume by genetic status

Years to disease onset (baseline)	**Symptomatic (n=18)**		**Preclinical (n=46) and Progressors (n=3)**		**Non-carriers (n=56)**	
	Mean (SD)	**CI**	**Mean (SD)**	**CI**	**Mean (SD)**	**CI**
Less than -20	-	-	1.74 (7.74)	-2.10, 5.59	-2.31 (6.83)	-5.71, 1.08
-20 to -10.01	-	-	3.28 (2.94)	1.18, 5.38	0.10 (3.04)	-2.44, 2.64
-10 to -0.1	-	-	6.88 (9.59)	0.79, 12.98	1.50 (3.92)	-0.99, 3.99
0 to 9.99	11.01 (7.86)	6.26, 15.76	5.96 (7.52)	0.18, 11.74	3.70 (4.65)	0.58, 6.82
10 and beyond	5.91 (4.50)	0.33, 11.50	-	-	3.38 (2.08)	1.45, 5.30

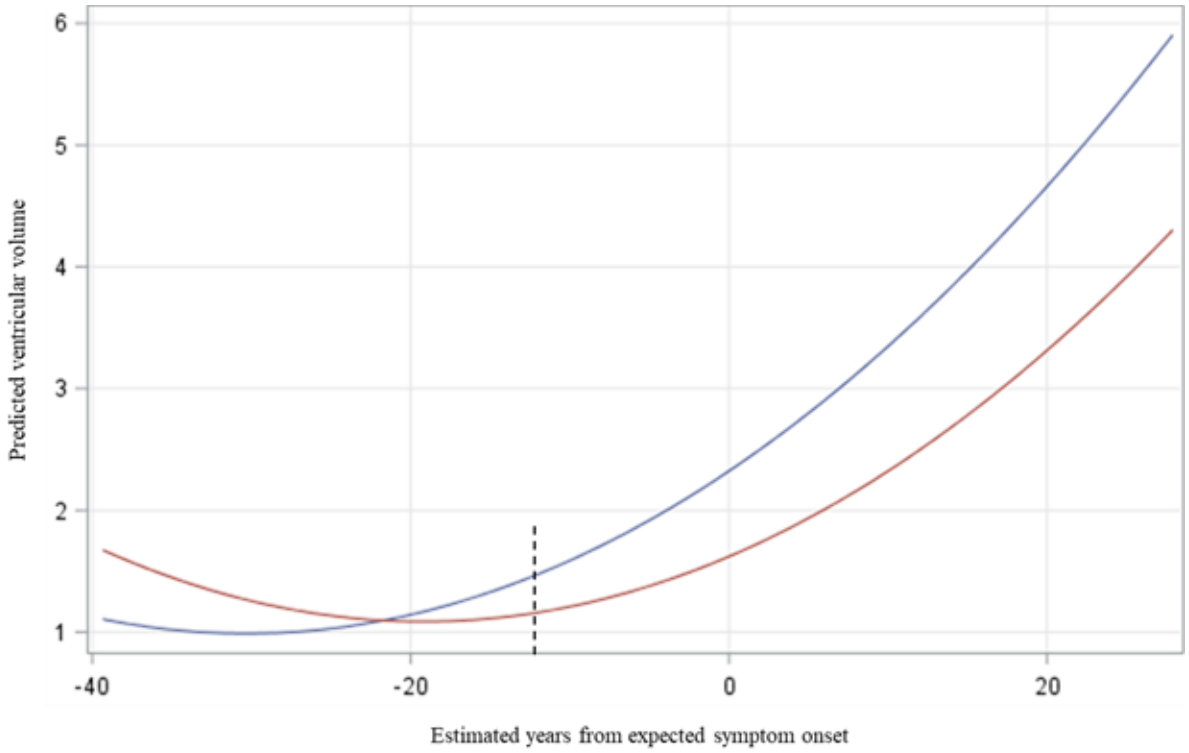
SD= Standard deviation; CI= 95% confidence interval
Years to disease onset for symptomatic individuals and progressors are based on actual time at diagnosis.

Table B.7. Annual percent change of total ventricular volume by genetic status

Years to symptom Onset[+]	**Symptomatic (n=18)**		**Preclinical (n=46) and Progressors (n=3)**		**Non-carriers (n=56)**	
	Mean (SD)	**CI**	**Mean (SD)**	**CI**	**Mean (SD)**	**CI**
Less than -20	-	-	1.76 (7.62)	-2.03, 5.55	-2.33 (6.89)	-5.76, 1.09
-20 to -10.01	-	-	3.39 (2.88)	1.32, 5.45	0.01 (2.91)	2.91, 2.44
-10 to -0.1	-	-	7.27 (10.33)	0.71, 13.83	1.52 (3.85)	-0.92, 3.97
0 to 9.99	10.97 (7.75)	6.29, 15.66	5.68 (7.64)	-0.19, 11.55	3.69 (4.58)	0.61, 6.77
10 and beyond	6.00 (4.47)	0.45, 11.56	-	-	3.15 (1.87)	1.42, 4.89

SD= Standard deviation; CI= 95% confidence intervals.
[+]Years to onset for symptomatic individuals and progressors are based on actual time at diagnosis.

Figure B.1 Predicted total ventricular volume by estimated years from expected disease onset in carriers (blue, n=67) and non-carriers (red, n=55) no extreme case.



Ventricular volume is expressed as a percentage of intracranial volume. To prevent disclosure of genetic status, individual data points are not plotted. Differences are noted beginning at 12 years prior to disease onset as indicated by the dashed line (p=0.05).

Table B. 8: Influence Diagnostic Statistics of High-Influential Participants (non-carriers)

Participant	PRESS Statistic	Cook's D	MDFFITS	COVRATIO	Restricted Likelihood Distance
1	22.93	0.12	0.13	0.61	1.66
2	3.84	0.38	0.34	0.86	2.92

Note: Model does not include the genetic status*time to disease onset quadratic term

Table B.9a: Estimates for preclinical carriers (n=46) and non-carriers (n=53) for *unedited* ventricular volumes with no extreme cases (n=1) or influential cases (n=2)

Fixed Effects	**Estimate**	**SE**	***p*-value**	**CI (95%)**
Intercept	0.35	0.35	0.31	-0.34, 1.05
Visit (ref=follow-up)	-0.0005	0.01	0.96	-0.02, 0.02
GS (ref=non-carrier)	0.36	0.15	0.02*	0.06, 0.67
Time from disease onset	-0.03	0.01	0.06*	-0.05, 0.0009
Time from disease onset^2	0.0008	0.0002	0.0001*	0.0004, 0.001
Time from disease onset*GS	0.02	0.008	0.02*	0.003, 0.03
Random Effects	**Estimate**	**SE**	***p*-Value**	
Family Membership	0.02	0.04	0.33	
Participant	0.38	0.07	<.0001*	
Residual	0.004	0.0005	<.0001*	

SE=standard error; GS=Genetic Status (carrier vs. non-carrier); *****significant at $p<0.05$

Table B.9b: Estimates between preclinical mutation carriers (n=46) and non-carriers (n=53) for *unedited* total ventricular volume by time to expected disease onset

	-25 years	-20 years	-15 years	-10 years	-5 years	0 years	5 years	10 years
Estimate	-0.10	-0.01	0.08	0.18	0.27	0.36	0.45	0.55
SE	0.16	0.14	0.13	0.13	0.14	0.15	0.18	0.20
***p*-value**	0.54	0.96	0.52	0.17	0.05	0.02*	0.01*	0.009*

SE=standard error; *****significant at $p<0.05$

Results section 2.0: Assessing regions of the ventricles for all mutation carriers vs. non-carriers, all preclinical carriers vs. non-carriers

Carriers vs. non-carriers: Regions of the Ventricles

Upon finding that total ventricular volume showed significant differences as a function of genetic carrier status and time to expected disease onset, we were then interested in exploring whether regions of the ventricles showed significant changes prior to disease onset using the same statistical model for volumes of the left ventricle, right ventricle, third and fourth ventricles. For each region, when significant, the interaction between genetic status and years from disease onset was followed up with *t*-tests. Relative to non-carriers, carriers (with extreme case removed) showed greater ventricle volumes in the left ventricle beginning 12 years prior to expected disease onset, in the right ventricle at 8 years, and in the third ventricle 17 years prior to disease onset. There was no significant interaction between genetic status and years from disease onset for the fourth ventricle or the laterality index.

Preclinical carriers vs. non-carriers: Regions of the Ventricles

Follow up analysis of left, right, third and fourth ventricular volumes with the final model with the two high-influential (n=2) and extreme case (n=1) removed demonstrated significant differences in the left ventricle beginning at 5 years before expected disease onset, and at 2 years *after* disease onset in the right ventricle. There was no significant genetic status by time to onset interaction for the third and fourth ventricles, and the laterality index.

Mean Ventricular Volumes: Manually Edited Versus Fully Automated Segmentations

We were interested in examining whether there were any significance differences between the constructed total ventricular volumes based on manual edits or unedited volumes.

Due to the potential differences in the degree of manual editing, differences were assessed separately at each visit (baseline and follow-up) for each group (symptomatic carriers, preclinical carriers, and non-carriers). Due to the small number of preclinical carriers who became symptomatic at follow-up (n=3), these individuals were not included in the following analysis. Normality was assessed using the Shapiro-Wilk and Kolmogorov-Smirnov tests using the Proc univariate procedure. When normality was violated, a Wilcoxon Signed-Rank test was conducted, otherwise a paired-samples t-test was used.

There were no differences between the edited and unedited volumes at baseline or follow-up for preclinical carriers (all p's> 0.80) and non-carriers (all p's> 0.146). For symptomatic carriers at baseline and follow-up, the edited (Baseline: M=3.037, SD=1.116; Follow-up: M=3.370, SD=1.230) and unedited (Baseline: M=3.058, SD=1.113; Follow-up: M=3.392, SD=1.227) volumes differed significantly.

Additionally, spearman's rank-order correlations were completed on total ventricular volume to delineate the relationship between the manual edited and unedited volumes for each group. All correlations were significant across all groups: all p's<0.0001, all r_s>0.99.

Appendix C: Chapter 4 (Study 3) Supplementary Material

Method C.1 Statistical Analysis for Voxel-based Morphometry Co-variate and Contrast
To account for grey matter volume differences influencing the fMRI signal, voxel-wise grey matter tissue probabilities were entered as a covariate in the fMRI analysis. Grey-matter probability maps were acquired using Statistical Parametric Mapping software (SPM12; Wellcome Department of Cognitive Neurology, London, UK; http://www.fil.ion.ucl.ac.uk/spm). T1-weighted images were converted into NifTI format using the *3dAFNItoNIFTI* command in AFNI. Next, T1-images were visually inspected and manually realigned to the anterior commissure to ensure proper spatial normalization. Following realignment, the images were segmented into grey and white matter and normalized to MNI space using the specifications of the AFNI @SSwarper MNI template (voxel size: 1x1x1; bounding box: Xstart: -96.5, Xend: 96.5, ystart: -114.5, yend:114.5, zstart:-96.5, zend: 96.5; smoothing: 4mm full-width at half-maximum Gaussian kernal). In AFNI, *3dresample* was run to ensure the orientation, dimensions and voxel size were identical between the resultant VBM images (smwc1.nii) and the participant's fMRI statistics file. Subsequently, the resampled grey-matter probability maps were included into the fMRI analysis as a voxel-wise covariate using 3dMVM in AFNI as a between subject factor.

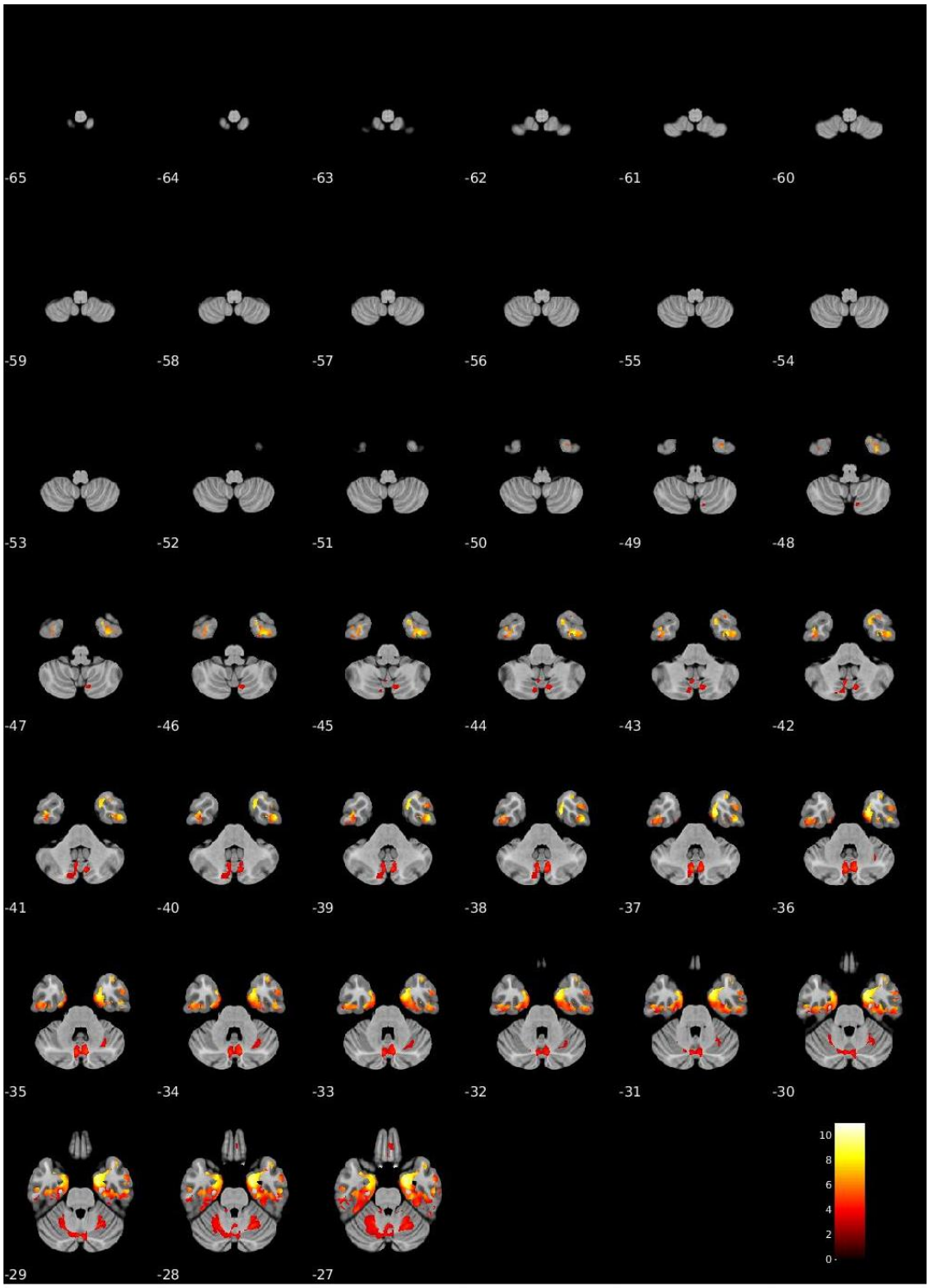
Multiple linear regression was conducted to assess regions that showed grey matter volume differences between patients with FTD and controls. Individual variation in brain size was accounted for by including total intracranial volume (sum of grey matter, white matter, and cerebrospinal fluid) as a covariate. AFNI's 3dFWHMx and 3dClustSim was used to obtain a cluster-size threshold at $p=0.05$, applied to the whole brain at an alpha threshold of $p<0.001$ (478 contiguous voxels).

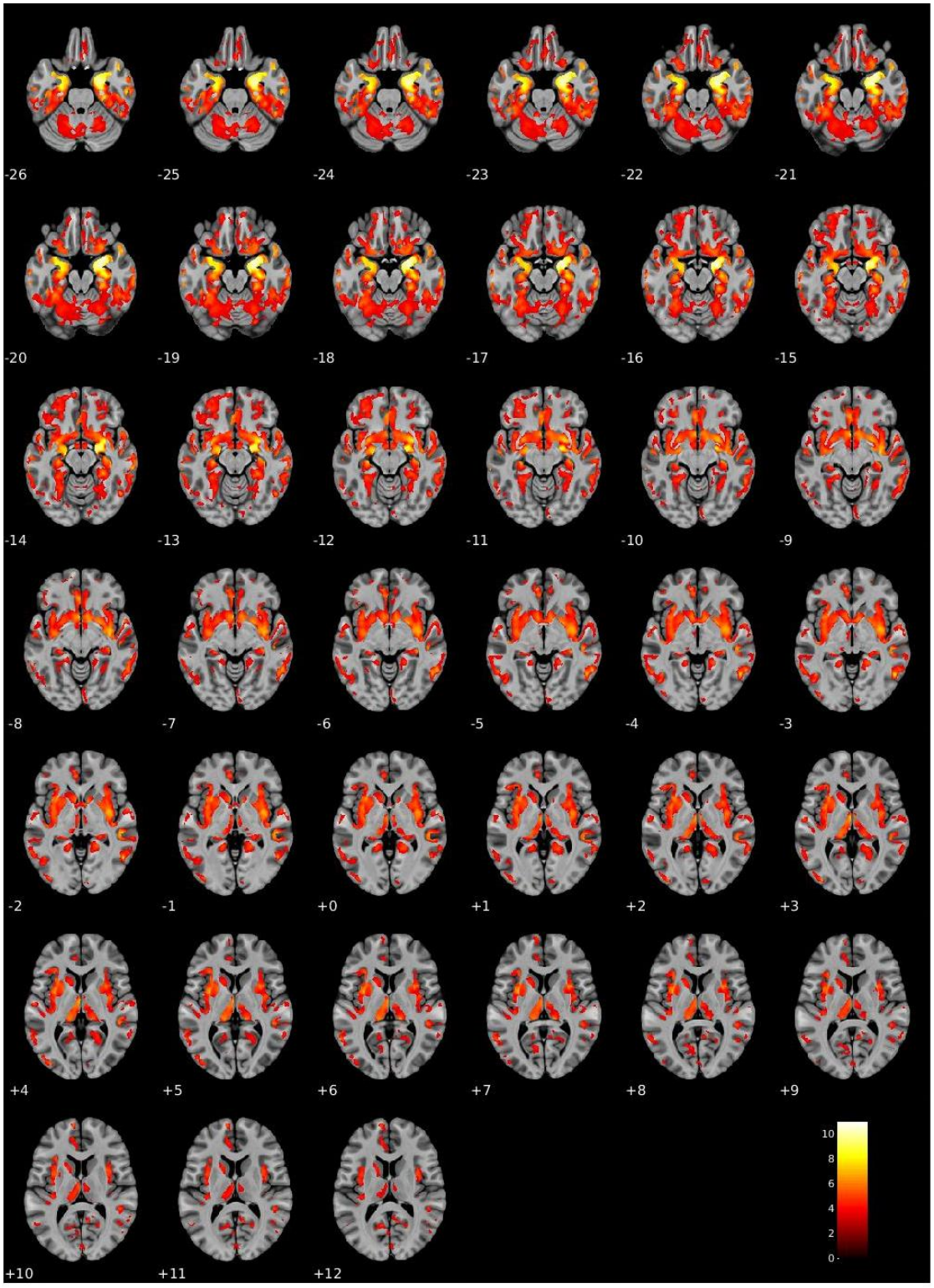
Table C.1 Grey Matter Volume Differences Between Controls and Patients with FTD

Cluster	Region	Cluster size (voxels)	t-statistic	X	Y	Z
1	Cluster expands across left and right frontal, temporal lobes, basal ganglia and cerebellum	142075	10.97	30	6	-22
2	Right middle temporal gyrus	809	7.26	45	9	-36
3	Left middle occipital gyrus	751	6.29	-38	-88	3
4	Left superior and middle temporal gyrus	2305	6.17	-51	7	-25
5	Left superior & medial frontal gyrus	776	5.64	-10	64	11
6	Right & left cuneus	2545	5.35	1	-87	16
7	Right inferior parietal lobe	640	5.15	34	-46	42
8	Left middle & inferior frontal gyrus	1050	5.06	-40	4	31
9	Right superior & inferior parietal lobe	520	5.00	64	-37	23
10	Left inferior parietal lobe	614	5.00	-62	-41	39
11	Right superior and middle temporal gyrus and precuneus	1336	4.57	51	-72	26

Voxel coordinates are in mm after transformation into standard MNI space.
Patients with FTD demonstrate less grey matter volume in clusters above

Figure C.1 Grey Matter Volume Differences Between Patients and Controls

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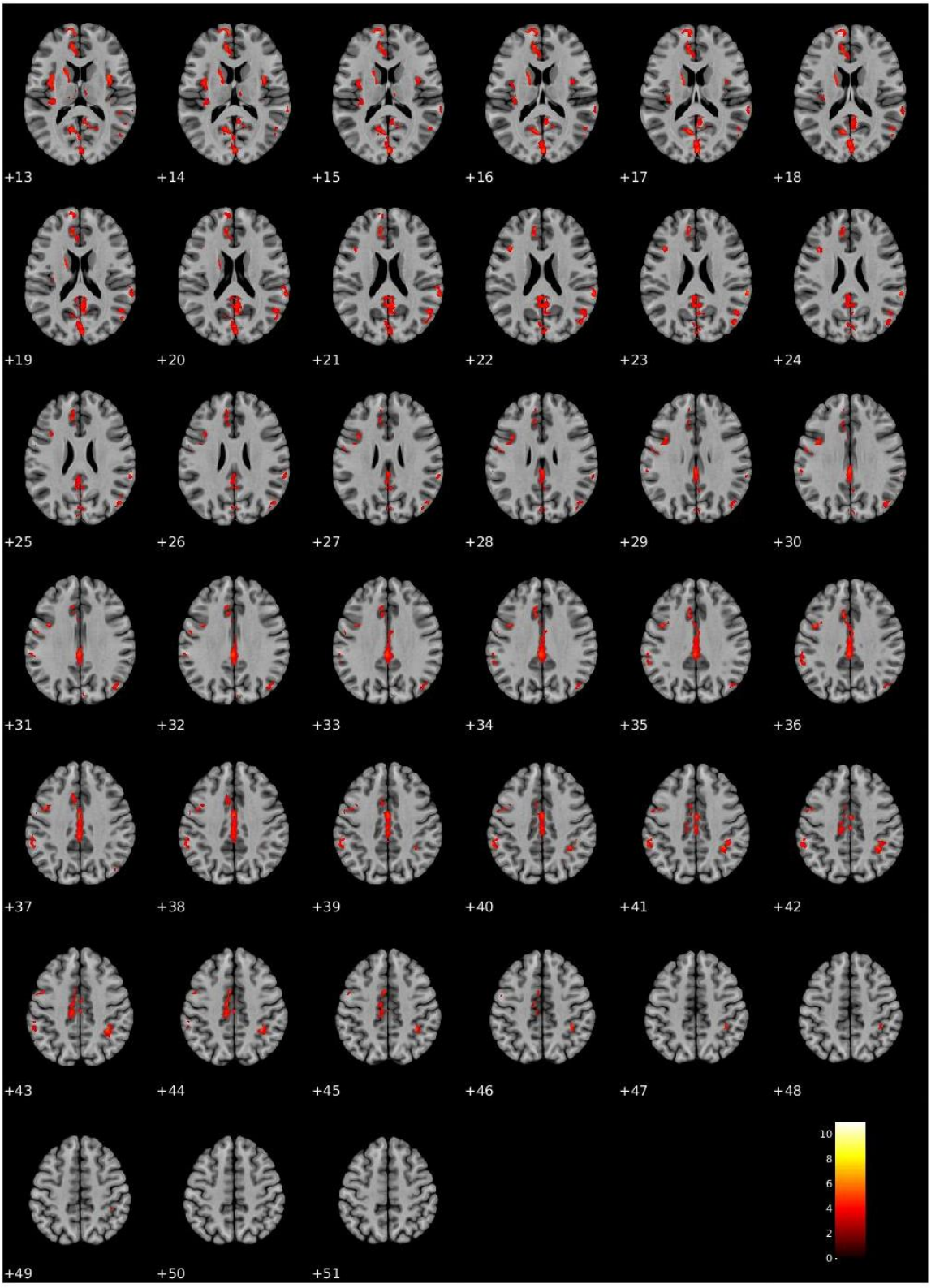
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Table C. 2. BOLD Signal Responses While Controlling for Grey Matter Volume

Choice Phase									
Fixed Effects	**Region**	**L/R**	**BA**	**X**	**Y**	**Z**	**t-value**	**# voxels**	**Contrast**
Group	Inferior frontal gyrus	L	47	32	-28.8	0.9	-3.0	53	Ctrl > Pat
	Middle temporal gyrus	L	22	60.4	31.9	3.7	-3.8	50	Ctrl > Pat
	Inferior parietal lobule	R	40	-54.0	47.0	27.4	-4.0	23	Ctrl > Pat
Phase	Inferior frontal gyrus	L	9	43.5	-5.2	34.5	3.1	190	Acq >Rev
	Medial frontal gyrus	L	6	5.2	-13.5	51.5	5.0	168	Acq >Rev
	Lentiform nucleus/putamen	L		16.2	-10.2	0.2	3.8	159	Acq >Rev
	Inferior frontal gyrus	R	9	-39.9	-6.6	31.0	4.3	95	Acq >Rev
	Cingulate gyrus	R	32	-9.2	-18.7	39.1	3.9	67	Acq >Rev
	Inferior frontal gyrus	L	47	32.0	-26.7	-4.6	4.7	61	Acq >Rev
	Caudate/lateral globus pallidus	R		-11.8	-6.8	1.3	3.4	36	Acq >Rev
	Inferior parietal lobule	L	40	52.8	45.4	49.2	5.6	23	Acq >Rev
	Cingulate gyrus	L	32	10.2	-16.5	34.4	4.8	22	Acq >Rev
Phase x reinforcement	Medial frontal gyrus	L	8/32	4.1	-19.8	47.9	-4.5	185	Acquisition: Incorr> Corr Reversal: Corr >Incorr
	Inferior frontal grus	L	9	44.0	-3.9	35.3	-3.8	155	Acquisition: Incorrrr > Corrr Reversal: Corr>Incorr
	Lentiform nucleus/putamen	L		+16.1	-10.4	-0.3	-3.2	125	Acquisition: Incorr> Corr Reversal: Corr>Incorr
	Inferior frontal gyrus	L	47	+35.0	-26.0	-5.0	-4.8	63	Acquisition: Incorr> Corr Reversal: Corr>Incorr
	Cingulate gyrus	R	32	-7.9	-17.6	42.2	-5.3	46	Acquisition: Incorr> Corr Reversal: Corr>Incorr
	Middle frontal gyrus	L	46	+43.0	-28.8	21.9	-4.7	36	Acquisition: Incorr> Corr Reversal: Corr>Incorr

	Cingulate gyrus	L	32	+10.4	-17.9	33.4	-4.2	27	Acquisition: Incorr > Corr Reversal: Corr >Incorr
Feedback Phase									
Fixed Effects	**Region**	**L/R**	**BA**	**X**	**Y**	**Z**	**t-value**	**# voxels**	
Reinforcement	Lateral prefrontal cortex	R		-29.5	-27.3	32.1	0.6	8468	Incorr >Corr
	Inferior parietal lobule	L	40	34.6	59.6	40.2	-6.2	4584	Incorr >Corr
	Lateral prefrontal cortex	L		42.9	-21.1	23.3	-4.4	4396	Incorr >Corr
	Inferior parietal lobule	R	40	-45.9	55.6	39.5	-4.5	3335	Incorr >Corr
	Cuneus	R	17	-1.3	82.8	6.6	-3.9	896	Incorr >Corr
	Fusiform gyrus	R	19	-44.3	70.3	-18.3	-4.0	493	Incorr >Corr
	Declive	L		30.7	63.3	-29.6	-3.9	474	Incorr >Corr
	Thalamus	R		-0.8	7.3	9.2	-3.3	349	Incorr >Corr
	Fusiform gyrus	L	19	45.4	71.0	-16.6	-3.3	145	Incorr >Corr
	Cingulate gyrus	L	23	2.0	24.4	30.5	-3.0	118	Incorr >Corr
	Posterior cingulate	L	30	19.7	63.8	3.8	-3.9	107	Incorr >Corr
	Declive	R		-12.0	82.0	-23.8	-4.4	95	Incorr >Corr
	Superior frontal gyrus	R	11	-21.8	-45.8	-15.6	-4.7	87	Incorr >Corr
	Middle occipital gyrus	L	19	34.5	91.5	6.7	-4.1	45	Incorr >Corr
	Middle temporal gyrus	R	21/22	-47.4	28.3	-5.4	-4.5	42	Incorr >Corr
	Middle frontal gyrus	L	11	23.8	-41.0	-16.5	-5.3	40	Incorr >Corr
	Lingual gyrus	R	18	-7.9	75.4	-7.9	-3.0	33	Incorr >Corr
	Middle occipital gyrus	L	19	44.3	59.6	-8.5	-3.5	32	Incorr >Corr
	Medial frontal gyrus	R	6	-21.3	-2.5	53.6	-4.6	29	Incorr >Corr
	Parahippocampal gyrus	L	27	8.9	37.4	4.6	-4.8	23	Incorr >Corr
	Caudate tail	R		-25.7	46.0	16.5	4.7	23	Corr > Incorr
Phase	Fusiform gyrus	R	19	-41.8	80.2	-21.0	3.8	83	Acq >Rev
	Inferior occipital gyrus	R	18	-28.8	90	-19.4	3.7	52	Acq >Rev
	Middle occipital gyrus	L	19	54.4	75.1	-1.7	4.3	42	Acq >Rev
	Lingual gyrus	L	18	3.7	79.6	-8.5	3.3	25	Acq >Rev

Group x Phase	Superior frontal gyrus	L	10/11	27.8	-56.1	-11.1	-5.8	50	Acquisition: Ctrl > Pat Reversal: Pat >Ctrl
Phase x Reinforcement	Middle occipital gyrus	R	19	-47.1	78.5	-10.7	-3.8	87	Acquisition: Incorr >Corr
	Inferior occipital gyrus	R	18	-26.4	90.1	-19.7	-4.4	63	Acquisition: Incorr >Corr
	Lingual gyrus	L	18	3.8	78.7	-8.7	-3.7	54	Acquisition: Incorr >Corr
	Cuneus	R	18	-4.5	97.2	8.9	-4.6	54	Acquisition: Incorr >Corr
	Cuneus	R	19	-0.8	89.5	27.6	-4.2	37	Acquisition: Incorr >Corr
	Fusiform gyrus	R	19	-42.9	78.5	-22.9	-4.6	25	Acquisition: Incorr >Corr
3-way interaction	Superior frontal gyrus	L	10/11	26.2	-56.1	-11.0	5.9	22	Acquisition Incorr: Ctrl > Pat Reversal Corr: Pat > Ctrl Reversal Incorr: Pat >Ctrl

Thresholded at $p<0.001$. All clusters survive correction for multiple comparison at $p<0.05$. Table displays regions, hemisphere (L, left; R, right), Brodmann area (BA), Montreal Neurological Institute (MNI) coordinates (x, y, z) at center of mass, maximum neural activity for the cluster (*t*-value), cluster size in voxels, and the direction of activity [Control (ctrl), Patient (pat), Acquisition (acq), Reversal (rev), Correct (corr), Incorrect (incorr)]. Focus point and regions of BA according to TT_Daemon: Talairach-Tournoux atlas. The ANOVA was completed separately for the choice and feedback phase.

Table C.3. Mean Percent Correct Responses and No Responses

	Acquisition Correct (SD)	Reversal Correct (SD)	Acquisition No Responses	Reversal No Responses
Patients	83.7 (15)	80.8 (16.3)	5.1 (8.8)	3.7 (10.3)
Controls	93.9 (2.2)	89.8(4.1)	0.3 (0.8)	0.2 (0.5)

Appendix D: Research Ethics Approval for Study I and II

Western Research **Use of Human Participants - Ethics Approval Notice**

Research Ethics

Principal Investigator: Elizabeth Finger
File Number:102510
Review Level: Full Board
Approved Local Adult Participants:15
Approved Local Minor Participants:0
Protocol Title: The GENetic Frontotemporal dementia Initative (GENFI): A new multi-centre platform for the study of frontotemporal lobar degeneration
Department & Institution: Schulich School of Medicine and Dentistry\Clinical Neurological Sciences,London Health Sciences Centre
Sponsor: Canadian Institutes of Health Research

Ethics Approval Date: September 04, 2012
Ethics Expiry Date: May 31, 2014

Documents Reviewed & Approved & Documents Received for Information:

Document Name	Comments	Version Date
Western University Protocol	(including instruments noted in section 8.1)	
Letter of Information & Consent	Patient with FTD	2012/07/09
Letter of Information & Consent	Family Member of Patient with FTD	2012/07/09
Caregiver Letter of Information & Consent		2012/07/09
Letter of Information & Consent	Lumbar Puncture	2012/07/09

This is to notify you that the University of Western Ontario Health Sciences Research Ethics Board (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced study on the approval date noted above. The membership of this HSREB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the University of Western Ontario Updated Approval Request form.

Member of the HSREB that are named as investigators in research studies, or declare a conflict of interest, do not participate in discussions related to, nor vote on, such studies when they are presented to the HSREB.

The Chair of the HSREB is Dr. Joseph Gilbert. The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Western Research

Date: 25 March 2020

To: Dr. Elizabeth Finger

Project ID: 102510

Study Title: The GENetic Frontotemporal dementia Initative (GENFI): A new multi-centre platform for the study of frontotemporal lobar degeneration (REB #18873)

Application Type: Continuing Ethics Review (CER) Form

Review Type: Delegated

REB Meeting Date: 07/Apr/2020

Date Approval Issued: 25/Mar/2020

REB Approval Expiry Date: 17/Apr/2021

Dear Dr. Elizabeth Finger,

The Western University Research Ethics Board has reviewed the application. This study, including all currently approved documents, has been re-approved until the expiry date noted above.

REB members involved in the research project do not participate in the review, discussion or decision.

Western University REB operates in compliance with, and is constituted in accordance with, the requirements of the TriCouncil Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The REB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

Sincerely,

Daniel Wyzynski, Research Ethics Coordinator, on behalf of Dr. Joseph Gilbert, HSREB Chair

Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).

Appendix E: Research Ethics Approval for Study III

Office of Research Ethics
The University of Western Ontario
Room 00045 Dental Sciences Building, London, ON, Canada N6A 5C1
Telephone: (519) 661-3036 Fax: (519) 850-2466 Email: ethics@uwo.ca
Website: www.uwo.ca/research/ethics

Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. E. Finger
Review Number: 13734 **Review Level:** Full Board
Review Date: November 6, 2007
Protocol Title: Investigating the Neurobiologic Etiologies of Behavioural and Cognitive Impairments in Frontotemporal Dementia
Department and Institution: Neurology, London Health Sciences Centre
Sponsor:
Ethics Approval Date: January 11, 2008 **Expiry Date:** October 31, 2012
Documents Reviewed and Approved: UWO Protocol, Letter of Information and Consent-Healthy Volunteers dated Dec 20, 2007, Letter of Information and Consent-Patients/Fam Members dated Dec 20, 2007, Letter of Information and Consent-Genetic Testing-Healthy Volunteers dated Dec 20, 2007, Letter of Information and Consent-Genetic Testing-Patient/Fam Members dated Dec 20, 2007
Documents Received for Information:

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced study on the approval date noted above. The membership of this REB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request Form.

During the course of the research, no deviations from, or changes to, the protocol or consent form may be initiated without prior written approval from the HSREB except when necessary to eliminate immediate hazards to the subject or when the change(s) involve only logistical or administrative aspects of the study (e.g. change of monitor, telephone number). Expedited review of minor change(s) in ongoing studies will be considered. Subjects must receive a copy of the signed information/consent documentation.

Investigators must promptly also report to the HSREB:

a) changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) all adverse and unexpected experiences or events that are both serious and unexpected;
c) new information that may adversely affect the safety of the subjects or the conduct of the study.

If these changes/adverse events require a change to the information/consent documentation, and/or recruitment advertisement, the newly revised information/consent documentation, and/or advertisement, must be submitted to this office for approval.

Members of the HSREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussion related to, nor vote on, such studies when they are presented to the HSREB.

Western Research

Date: 11 October 2019

To: Elizabeth Finger

Project ID: 4811

Study Title: Investigating the Neurobiologic Etiologies of Behavioural and Cognitive Impairments in Neurodegenerative Dementia (REB #13734)

Application Type: Continuing Ethics Review (CER) Form

Review Type: Delegated

REB Meeting Date: 15/Oct/2019

Date Approval Issued: 11/Oct/2019

REB Approval Expiry Date: 06/Nov/2020

Dear Elizabeth Finger,

The Western University Research Ethics Board has reviewed the application. This study, including all currently approved documents, has been re-approved until the expiry date noted above.

REB members involved in the research project do not participate in the review, discussion or decision.

Western University REB operates in compliance with, and is constituted in accordance with, the requirements of the TriCouncil Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The REB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

Sincerely,

Daniel Wyzynski, Research Ethics Coordinator, on behalf of Dr. Joseph Gilbert, HSREB Chair

Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).

Curriculum Vitae

Name: Tamara P. Tavares

Post-secondary Education and Degrees:

University of Toronto, Mississauga
Mississauga, Ontario, Canada
2009 - 2013, HBSc

Western University
London, Ontario, Canada
2013 – 2020, Ph.D.

Honours and Awards:

Ontario Volunteer Service Award, 5 Year Recognition
2019

The Jonathan & Joshua Graduate Scholarship in Mental Health Research
2014-2015, 2015-2016, 2016-2017

Ontario Graduate Scholarship
2015-2016 (declined), 2016-2017 (declined)

Western's 3 Minute Thesis Final, 1st place
2017

Alexander Graham Bell Canada Graduate Scholarship-Doctoral
2016-2019

Doctoral Excellence Research Award
2016-2018

Western Graduate Research Scholarship, PhD Award
2015-2017

Schulich Graduate Scholarship
2015-2017

Western Graduate Research Scholarship, Masters Award
2013-2015

NSERC Undergraduate Student Research Award
2012-2013

University of Toronto Dean's Honour List
2010-2013

Related Word Experience

Teaching Assistant
Neuroscience for Rehabilitation Sciences (Anatomy & Cell Biology 9531)
University of Western Ontario
09/2014-12/2014

Teaching Assistant,
Introduction to Psychology (Psychology 1000)
University of Western Ontario
09/2013-04/2014

Publications:

Tavares TP, Kerr E, Smith ML. Memory Outcomes Following Hemispherectomy in Children. (2020, In press). *Epilepsy & Behaviour*. doi.org/10.1016/j.yebeh.2020.107360

Tavares TP, Mitchell D, Coleman K, Coleman BL, Shoesmith CL, Cash DM, Moore KM, van Swieten J, Borroni B, Galimberti D, Tartaglia MC, Rowe JB, Graff C, Tagliavini F, Frisoni G, Cappa S, Jr Laforce R, de Mendonca A, Sorbi S, Masellis M, Rohrer JD, Finger E. (2020, In press). Early Symptoms in Symptomatic and Preclinical Genetic Frontotemporal Lobar Degeneration. *Journal of Neurology, Neurosurgery and Psychiatry*. doi:10.1136/jnnp-2020-322987

Mitchell E, **Tavares TP**, Pallaniyappan L, Finger E. (2019). Hoarding and Obsessive-Compulsive Behaviors in Frontotemporal Dementia: Clinical and Neuroanatomic Associations. *Cortex,* Epub. doi.org/10.1016/j.cortex.2019.09.012

Tavares TP, Mitchell D, Coleman K, Shoesmith CL, Bartha R, Cash DM, Moore KM, van Swieten J, Borroni B, Galimberti D, Tartaglia MC, Rowe JB, Graff C, Tagliavini F, Frisoni G, Cappa S, Jr Laforce R, de Mendonca A, Sorbi S, Wallstrom G, Masellis M, Rohrer JD, Finger E. (2019). Ventricular Volume Expansion in Presymptomatic Genetic Frontotemporal Dementia. *Neurology 93*: e1-e8. doi:10.1212/WNL.0000000000008386

Puka K, **Tavares TP**, Speechley KN. (2019). Social Outcomes of Adults with Childhood-onset Epilepsy: A Systematic Review and Meta-analysis. *Epilepsy and Behavior 92,* 297-305. doi.org/10.1016/j.yebeh.2019.01.012

Puka K, **Tavares TP**, Anderson K, Ferro M, Speechley KN. (2018). A Systematic Review of Quality of Life in Parents of Children with Epilepsy. *Epilepsy and Behaviour 81, 38-45.* doi.org/10.1016/j.yebeh.2018.03.008

Puka K, **Tavares TP**, Smith ML. (2017). Development of Intelligence 4 to 11 Years after Pediatric Epilepsy Surgery. *Journal of Neuropsychology 11(1),* 161-173. doi: 10.1111/jnp.12081

Vieira JB, **Tavares TP**, Marsh A, Mitchell DGV. (2017). Emotion and Personal Space: Neural Correlates of Approach-Avoidance Tendencies to Different Facial Expressions as a Function of Coldhearted Psychopathic Traits. *Human Brain Mapping, 38 (3),* 1492-1506. doi:10.1002/hbm.23467

Tavares TP, Logie K, Mitchell DGV. (2016). Opposing Effects of Perceptual versus Working Memory Load on Emotional Distraction.

Experimental Brain Research, 234 (10), 2945-2956. doi: 10.1007/s00221-016-4697-2

Tavares TP, Puka K, Smith ML. (2015). Emotional Functioning: Long-term Outcomes after Pediatric Epilepsy Surgery, *Epilepsia, 56*(5), 745-753. doi: 10.1111/epi.12956

Book Chapters

Tavares TP. (2019). Social-Cognitive Perspective. In: Zeigler-Hill V., Shackelford T. (eds) Encyclopedia of Personality and Individual Differences. Springer, Cham. doi.org/10.1007/978-3-319-28099-8_1844-1

Tavares TP (2018) Case Study. In: Zeigler-Hill V., Shackelford T. (eds) Encyclopedia of Personality and Individual Differences. Springer, Cham doi.org/10.1007/978-3-319-28099-8

Tavares TP (2017) Electroencephalography (EEG). In: Zeigler-Hill V., Shackelford T. (eds) Encyclopedia of Personality and Individual Differences. Springer, Cham. doi.org/10.1007/978-3-319-28099-8

Tavares TP (2017) Medial Prefrontal Cortex (MedPFC In: Zeigler-Hill V., Shackelford T. (eds) Encyclopedia of Personality and Individual Differences. Springer, Cham. doi.org/10.1007/978-3-319-28099-8